KINETICS OF COMPLETELY-MIXED ACTIVATED SLUDGE

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CHAPTER I

INTRODUCTION

Man has always been interested in improving his environment so that he could live a happier, healthier, and better life. He realized the important part played by water, and developed techniques and methods to preserve the water resources in the world. However, with increasing population and industrialization the water requirements have increased more than proportionally, and soon he will be facing the problem of insufficient water of desired quality, and greater reliance will have to be placed upon treatment and reuse of the waste water.

To get a better insight into the problem, let us review the situation in the United States. In this country the natural runoff from rain and snow amounts to approximately 1250 billion gallons a day, of which only about 650 billion gallons are recoverable by existing methods. The present water requirements amount to approximately 360 billion gallons a day. Even though there is a surplus quantity of water at the present time, the water requirements are expected to increase to 650 billion gallons a day by the year 1980. And by the year 2000 the

water requirements will increase to 1000 billion gallons a day, which is considerably higher than the available water (1). In order to meet the future demands it has become extremely important to find new sources of water.

The technological development of desalination processes has shown rapid progress in recent years. However, the prohibitory costs of converting sea water make it impracticable for large scale application. Conversion of sea water costs approximately 40 cents to one dollar per 1000 gallons, depending upon the conversion process employed (2). This is comparatively higher than the present national average cost of 13 cents per 1000 gallons.

It seems probable that the increased reuse of water will be the only practical means for supplying the future water needs. When it becomes necessary, the used water has to be so treated that its repeated use will not deteriorate its quality. Even at present, pollution of natural bodies of water is the major cause of water shortages in many cities. A prescient Senate committee stated in 1960: "Where the municipalities run out of water, it will not be because of a lack of water but because the water is unfit for use." (1). The frequent introduction of new organic compounds by industries and the so-called "refractory contaminants" present a constant challenge to waste water treatment engineers. Unless new methods of treatment are developed or the present methods are modi-

fied, the natural bodies of water will soon become a chain of open sewers. Therefore, it has become increasingly important to improve waste treatment practices so that the pollutants entering natural waters will be reduced or even eliminated.

One of the major signs of progress is the recent realization that the activated sludge process resembles an open continuous fermentation process. Even though activated sludge processes have been operated successfully for over half a century, they have been designed and operated on a purely empirical basis. The empirical formulations were derived mainly from studies employing batch fed units. While such units are suitable for acclimation purposes, they do not simulate actual operating conditions of the prototype activated sludge process. Furthermore, the wide variations in strength and characteristics of industrial wastes militate against the successful use of "rule of thumb" procedures in design. The situation has been summed up by Wuhrmann (3): "There is too much engineering and too little microbiology in the waste treatment practice of today." The realization of similarities between the activated sludge process and other continuous fermentation processes could help provide basic knowledge about the waste water treatment process. The systematic procedures, nomenclature, and mathematical treatment of processes used in fermentation technology could be appli-

cable to waste treatment as well. Some of the advantages that have been claimed in favor of the completely-mixed continuous flow processes are:

(a) uniform organic loading: The aeration tank acts as a surge tank in minimizing the effect of both qualitative and quantitative shock loading

(b) maintenance of a uniform physiological state of the active organisms over an indefinite period

(c) uniform effluent characteristics

(d) ease of automatic control of the process

(e) elimination of pretreatment of certain toxic and alkaline wastes, and

(f) reduction in plant size for the same volume of waste.

While the above advantages of economy and efficiency favor the introduction of the process on a large scale, lack of basic information about its kinetics and design criteria retard its increased use. It is the primary object of this research to investigate the various kinetic relationships of the completely-mixed activated sludge process and to provide a mathematical expression to describe its behavior. Even though some of the steady state kinetic relationships have already been provided by basic microbiological and fermentation process research,

they may not be directly applicable to the waste treatment process. It is emphasized that the research in fermentation processes has been carried out for the most part with pure cultures. Because of the great potential that completely-mixed continuous flow reactors have for use in waste water treatment, it was felt that an extended study of their kinetic behavior was warranted. Because of the heterogeneity of the population, it was essential that massive amounts of data be collected on a wide variety of operational parameters. Long-term studies were required, since it was not known whether steady state conditions could be attained with heterogeneous populations.

In the present study continuous flow units were run at various dilution rates, with and without sludge recycling. "Steady state" values of substrate concentration, biological solids, pH, temperature, and carbohydrate concentration were determined at each flow rate. Inflow substrate concentrations of 1000 and 3000 mg/l glucose were used in the studies without sludge recycle. For experiments which included return sludge, only the 1000 mg/l substrate level was used. An extended study was also made with total recirculation of sludge. Microscopic examination of the cluture was made at each flow rate to gain an insight into the effect of flow rate on population dynamics.

It is believed that the process variables and kinetic relationships studied in this research will fulfill part of the requirements that would be necessary to describe the process mathematically. Two major difficulties have been listed by Herbert (4) for the general neglect of continuous culture studies: (a) lack of generally accepted theoretical background, and (b) general belief that they are so difficult as to be impracticable.

The research work presented herein is an attempt to overcome the above difficulties and to gain information which could ultimately permit enlightened and more theoretically satisfying design of the continuous flow activated sludge process.

CHAPTER II

THEORY OF CONTINUOUS FLOW PROCESS

1. General Description

All continuous flow processes consist essentially of a reactor into which there is a continuous addition of reactants at a predetermined flow rate. The products and unreacted materials emerge from the reactor at the same rate at which the reactants are introduced. A constant volume is maintained in the reactor by means of an overflow device. The continuous flow process is applicable either for purely chemical reactions or for biochemical reactions or for a combination of both. In the present study the major emphasis is placed on biochemical processes.

The biochemical process is initiated by introducing a nutrient solution into the reactor and inoculating it with a suitable culture of microorganisms. The conversion of nutrients to desired products is carried out in the reactor and the products are separated in subsequent operations. The desired product may be either the microorganisms themselves or other metabolic products. Certain examples of continuous flow processes in which

microorganisms carry out the reactions are:

(a) production of bakers' yeast, food yeast, chlorella, etc.

(b) production of ethanol, butanediol, and other metabolic products by continuous deep fermentation

(c) production of acetic acid in "vinegar towers"

(d) production of antibiotics and vitamins

(e) transformation of steroids

(f) disposal of industrial wastes, and

(g) various sewage treatment processes, including trickling filters, the activated sludge process, and sludge digestion.

Continuous flow processes may be classified according to the manner in which they are operated: (a) piston-flow, or tubular, reactors; (b) completely-mixed reactors; and (c) partially-mixed reactors.

(a) Piston-Flow or Tubular Reactors

Piston-flow reactors are operated in such a way as to maintain or approach streamlined flow conditions inside the reactor. The nutrient solution and the microorganisms are mixed before entering the reactor. The mixture passes through successive portions of the reactor without mixing. Each element of fluid stays in the reactor for the entire detention period. The deten-

tion period is calculated from the volume of the reactor (V), and the flow rate (F) of the reaction mixture. As the reaction mixture passes through the reactor, the nutrients undergo biochemical transformations, and the products are collected at the outlet end. A schematic representation of a piston-flow reactor is shown in Figure 1.

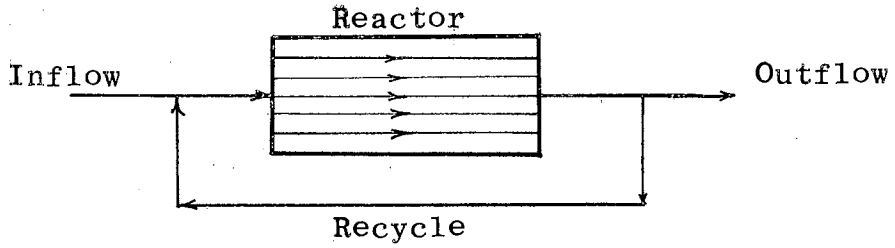

Figure 1. Schematic Representation of a Piston-Flow Reactor.

It can be easily visualized from the above figure that there is no mixing, particularly along the direction of flow. This suggests that the growth of the microbial population in a piston-flow reactor is identical to the growth of a batch-fed culture and undergoes part or all of the growth cycle. Therefore, depletion of nutrients and accumulation of toxic metabolites may impede the metabolic activity of the culture near the outlet end of the reactor. Because of these reasons the piston-flow reactors are more commonly used by the chemical industry than by microbiologists (5).

(b) Completely-Mixed Reactors

In the completely-mixed system the inflow nutrient is instantaneously and thoroughly mixed in the reactor. The mixing is accomplished either by impellers or by gas diffusion. In an ideal reactor the mixed liquor is uniform and homogeneous throughout, and the outflowing liquor will be identical in composition to the mixed liquor. If such a system is in the steady state, the microbial growth is in an exponential phase. A schematic representation of a completely-mixed system is shown in Figure 2. A completely-mixed system can be run with or without feedback or cell recycling, since an equilibrium is established between the microbial growth inside the reactor and the flow rate of the nutrient solution. Any change in the system parameters will result only in the shifting of the equilibrium position, but will never result in a permanent disturbance to equilibrium. This equilibrium has been referred to by many researchers as a "steady state" condition (6, 7, 8, 9, 10, 11, 12).

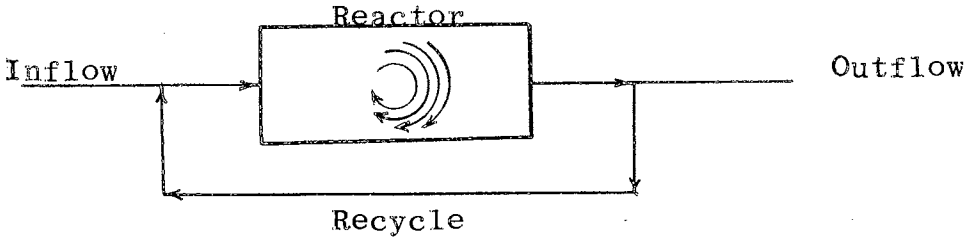

Figure 2. Schematic Representation of a Completely-Mixed Reactor.

Completely-mixed systems can be further classified as (a) internally controlled, and (b) externally controlled systems (13, 14). In the internally controlled system the rate of flow of nutrient (F) to the reactor changes according to the variations in the bacterial density. This is controlled by a sensing element such as a photo cell which detects internal changes in the system, such as bacterial density, pH, or chemical concentration. Thus the successful operation of an internally controlled system depends upon the sensitivity of the density detecting system. In any such system the organisms will grow at the maximum rate characterized by the environmental conditions in the reactor. The operator can select any desired bactrial density, but he can produce a change in the growth rate only by a change in nutrients, temperature, or pH.

In an externally controlled system the flow rate (F) is kept constant at some fixed value below the maximum growth rate. The growth medium contains an excess of all nutrients except one. The nutrient which is not in excess is the growth-limiting factor. Under these conditions the bacterial density will increase in the reactor; however, as the bacterial density increases, the feed/cell ratio will decrease and tend to become very small. Then the growth rate will begin to decrease. Soon an equilibrium at which the system parameters will not change with time is established. This system is thus said to be self-

stabilizing, and free from oscillations. Novick (13) has indicated that a wide variety of substances can be used as the growth-limiting factor. Among the growth limiting factors that have been employed are:

(a) a required amino acid (e.g., tryptophan, proline, histidine)

(b) the energy source or carbon source (e.g., lactose, glucose, maltose)

(c) an inorganic nutrient (e.g., ammonia, phosphate, magnesium sulfate).

A great advantage of externally controlled reactors is that they provide experimental systems in which not only is the growth-limiting factor known, but also, the concentration of the chosen growth-limiting factor can be varied in a known way.

(c) Partially-Mixed Systems

When shortcircuiting and imperfect mixing conditions occur in a completely-mixed system, it may be termed a "partially-mixed" system. This can be observed when the measured parameters in the effluent differ from those of the mixed liquor in the reactor. The factors that may induce imperfect mixing conditions in a continuous flow process are:

(a) geometry of the reactor (size and shape)

(b) amount of air supplied

(c) impeller speed

(d) size of the impeller relative to the size of the reactor, and

(e) type of microbial culture used in the study.

The last factor plays an important role in the operation of continuous flow processes. Adherence of cells to the walls of the reactor and filamentous growth of certain cultures, such as Sphaerotilus and molds, could create imperfect mixing conditions under otherwise perfect mechanical conditions. Steady state conditions cannot be maintained in a partially-mixed reactor for an indefinite period. A phenomenon known as "oscillation" will occur, and the performance of the process will become unpredictable. The term "oscillation" refers to fluctuations in the system parameters, such as the concentration of biological solids, chemical oxygen demand, etc. The agents causing oscillation in a completely-mixed reactor may be either physical or biochemical. The former can be controlled by proper design of the reactor so that the effluent and mixed liquor in the reactor are identical. Biochemical agents are beyond the limits of control, and variations are caused either by predominance of certain species over others or by changing yield coefficients. The combined effect of both the factors decides the extent of oscillation in studies involving heterogeneous populations. Hetling, et al. (15) have reported that the yield coefficients are not true constants, and vary widely

even for pure cultures utilizing a single organic compound as sole source of carbon. Rao and Gaudy (16) have also reported that the value of the yield coefficient varies between 48 and 82 per cent for activated sludge utilizing glucose as carbon source under controlled conditions. They have also concluded that the change in yield coefficient might be due to changes in predominance of the bacterial population. It appears from the above discussion that oscillation is inevitable in any long-term operation and plays an important role in the establishment of the kinetics of continuous flow processes.

Deindoerfer and Humphrey (17), and Herbert (4) have presented other types of classification of continuous flow processes based on (a) biochemical, and (b) chemical engineering principles.

The biochemical approach to classification takes into account the fact that biochemical transformations can be brought about either with accompanying growth of microorganisms or in the absence of growth. They are referred to as (a) growth, and (b) non-growth systems. In the process which involves growth, the bio-catalytic activity is a function of limiting nutrient concentration and the product formed is directly proportional to the cellular concentration attained as well as to the amount of limiting nutrient utilized. In the non-growth process, the suspended cell concentration provides the enzyme to con-

vert substrate to extracellular products in a manner analogous to the chemical reactions carried out by solid catalysts. Even resting cells can bring about the chemical transformation, provided the necessary enzymes are present. The latter process is also referred to as a "catabolic process" or breakdown to simpler molecules, and an example would be the breakdown of glucose to ethanol. The process is exergonic and can be brought about even by resting cells. The growth process has been termed as a "biosynthetic process," and an example would be the production of penicillin (4).

The chemical engineering classification offers several different criteria. One important distinction is between homogeneous and heterogeneous systems. In a homogeneous system, the composition of reactor liquid is uniform throughout. In the heterogeneous system, there may exist a concentration gradient of cells, or substrate, or any other, or all of the system parameters. Microorganisms passing through a heterogeneous reactor will undergo something akin to the growth cycle of a batch culture.

All continuous flow systems can be classified as either single phase or multiphase systems. In the single phase system the biochemical transformation takes place in one phase, generally the liquid phase. Single phase systems can be either homogeneous or heterogeneous. The multiphase system, as the name implies, involves more

than one phase. A typical example of a multiphase system is the trickling filter. The operation of trickling filters involves a solid and a liquid phase. The waste is passed through a filter bed during which time the microorganisms attached to the bed metabolize the organic substrates present in the waste. The efficiency of the process depends upon the extent of contact with the microorganisms. Multiphase reactors are necessarily heterogeneous systems.

Classifications such as "open" and "closed" systems have been presented by Herbert (4). In the closed system the microorganisms never leave the reactor and may be retained by means of a semipermeable membrane. Such a system is formally analogous to the so-called total oxidation or "total recirculation" system employed in waste water treatment in which there is no sludge wasting. Thus the continuous-flow process provides much flexibility in operation with uniform quality of end products over an indefinite period. Because of the uniformity of reaction products and reaction rates in continuous-flow completely-mixed reactors, they are more suitable for the study of biochemical reactions than fill-and-draw or batch process.

In the fill-and-draw or batch process the overall operational conditions are selected empirically for optimizing the various steps such as energy transfer, catalyst synthesis and product formation. This can result in the

inefficient operation of the process, since each of the above steps might require quite different optimum conditions. Continuous flow processes offer a striking advantage in this aspect, since a series of reactors can be used for optimizing each of the above steps.

Even though the continuous flow process appears to be far superior to batch systems, it does have disadvantages. The major disadvantage is that failure in any one of the unit operations will result in total failure of the system. When the process involves the use of pure cultures, contamination or generation of mutants will also result in the shutdown of the process. However, in the main, continuous flow processes are more promising than fill-and-draw operations for nearly all bioengineering systems and in particular for waste water treatment.

2. Kinetics of Continuous Flow Process

The successful application of any process rests on the establishment of various reaction rates and the relationships between different process variables. The complicated nature of biological processes has led to formulation of many different relationships describing the kinetics of the process. Among those worthy of mention here are the works of Monod (20), Spicer (9), Herbert, et al. (5), Garrett and Sawyer (18), and Moser (19). Since the mineralization of organic compounds is generally accompanied

by microbial growth, only the kinetics of growth systems will be discussed in the following section.

Kinetic study of any continuous culture system must take into account (a) the flow characteristics of the reactor system employed, and (b) the kinetics of microbial growth. The flow characteristics are determined by the rate of flow of influent nutrients and the degree of mixing. The kinetics of microbial growth follow the same biochemical principles whether a given system is designed for the formation of various desired saleable products or for waste water treatment. The object of waste water treatment is usually to minimize the adverse effects that wastes have on water courses where oxygen deficiency results in nuisance problems and the destruction of aquatic flora and fauna. An even more important objective, and one which is becoming more and more apparent, is to prepare the water for reuse. Therefore, the desired product is reusable water, and biological treatment of aqueous organic wastes is one of the key unit operations in this overall process. Stewart, et al. (21) have stated that the basic biochemical principles involved will be the same, whether the system is operated under aerobic or anaerobic conditions. The microorganisms involved in anaerobic fermentation are primarily bacteria which have the ability, in the absence of free oxygen, to decompose complex organic matter into the principal end products, methane and carbon dioxide.

(a) Flow Characteristics

In order to study the flow characteristics of a continuous flow process, it is proper to employ a system in which there are no side reactions taking place. The presence of microorganisms or any other chemicals in the reactor might alter the composition of inflow liquor and make it impossible to predict the hydraulic performance of the reactor system. Use of a dye or of salt solutions has been suggested and employed by previous research workers (22, 23). Let us consider a reactor of Volume V, filled initially with distilled water. A dye solution is then pumped in at a predetermined flow rate, F. The first case which will be examined is that of a piston-flow reactor.

Piston-Flow Reactor

Since there is no mixing in a tubular reactor other than by diffusion, the effluent will be distilled water until a period has passed equal (or nearly equal) to the detention time. After that period the reactor will be filled with dye solution, and the effluent from the reactor will exhibit the same concentration of dye as that of the inflow. The detention period is numerically equal to the ratio between the volume of the reactor (V) and the flow rate (F).

The same experiment can be conducted by initially filling the reactor with the dye solution and by pumping

in distilled water. Let C_0 be the concentration of dye solution in the reactor at the start. When the flow of distilled water is begun, the concentration of dye solution in the effluent will be constant and equal to C_0 for a period equal to the detention time. The variation in the concentration of dye (C) in the effluent at any time, t, is shown in Figure 3. Figure 3 is applicable only to an ideal reactor, in which all particles have a residence time equal to the mean residence time, $\bar{t}$.

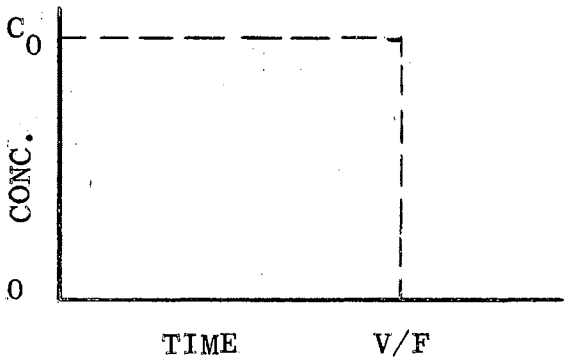

Figure 3. Flow Characteristics of a Piston-Flow Reactor.

$$\bar{t} = \frac{V}{F} = \frac{1}{D} \qquad (1)$$

where D is the "dilution rate." For any tubular reactor there will be a certain amount of mixing or axial dispersion, which will cause a spread of residence time about the mean. The residence time distribution curve will be a single peaked one, the frequency function $\phi(t)$ having the form of a Gaussian function (4).

Completely-Mixed Reactors

The flow characteristics of a completely-mixed reactor (Figure 2) can be determined in a manner similar to that described above. Let C_0 be the concentration of dye in the reactor at t equal to t_0, where t_0 refers to the initial time. Let C be the concentration of dye in the reactor at any time, t. Then

If $\frac{dC}{dt}$ is the rate of change of dye concentration,

$$\frac{dC}{dt} = \frac{F}{V} C_i - \frac{F}{V} C \qquad (2)$$

where C_i is the concentration of dye in the inflow and is a constant. From Equation 2

$$\frac{dC}{dt} = DC_i - DC = D(C_i - C)$$

where D is the dilution rate. The dilution rate D represents the number of complete turnovers per unit time. It can also be defined as the fraction of reactants or products that leaves the reactor per unit time.

Integrating the above expression between the limits C_0 and C when t = 0 and t = t respectively,

$$\text{Log}_e \left[\frac{C_i - C}{C_i - C_0}\right] = - Dt \qquad (3)$$

$$\left[\frac{C_i - C}{C_i - C_0}\right] = e^{-Dt} \qquad (4)$$

When the reactor is initially filled with water, and a dye is pumped in, Equation 4 reduces to

$$C = C_i (1 - e^{-Dt}), \qquad (5)$$

since $C_0 = 0$. When the reactor is initially filled with dye solution and water is pumped, Equation 2 upon integration will give

$$C = C_0 \, e^{-Dt}, \tag{6}$$

since $C_i = 0$. By plotting the theoretical curves from Equations 5 or 6, and observed values, the flow characteristics of a particular reactor can be evaluated. From Equations 5 and 6 it can be seen that only when t is equal to infinity does $C = C_i$, and $C = 0$, respectively. Theoretical plots of the Equations 5 and 6 are shown in Figure 4. The mean residence time of a completely mixed system will also be equal to $V/F = \bar{t}$, but the distribution of residence times will be entirely different from a piston-flow reactor. The distribution of residence times will be exponential with a frequency function

$$\phi(t) = D \, e^{-Dt} \tag{7}$$

where $\phi(t)$ is the frequency function.

(b) Kinetics of Microbial Growth

As has already been mentioned, microbial growth in a piston-flow reactor will be similar to growth in a batch culture. Therefore, attention will be given in this section to the growth of microorganisms in a completely-mixed system. Since the establishment of a steady state is an inherent characteristic of a completely-mixed continuous culture, only growth under steady state conditions

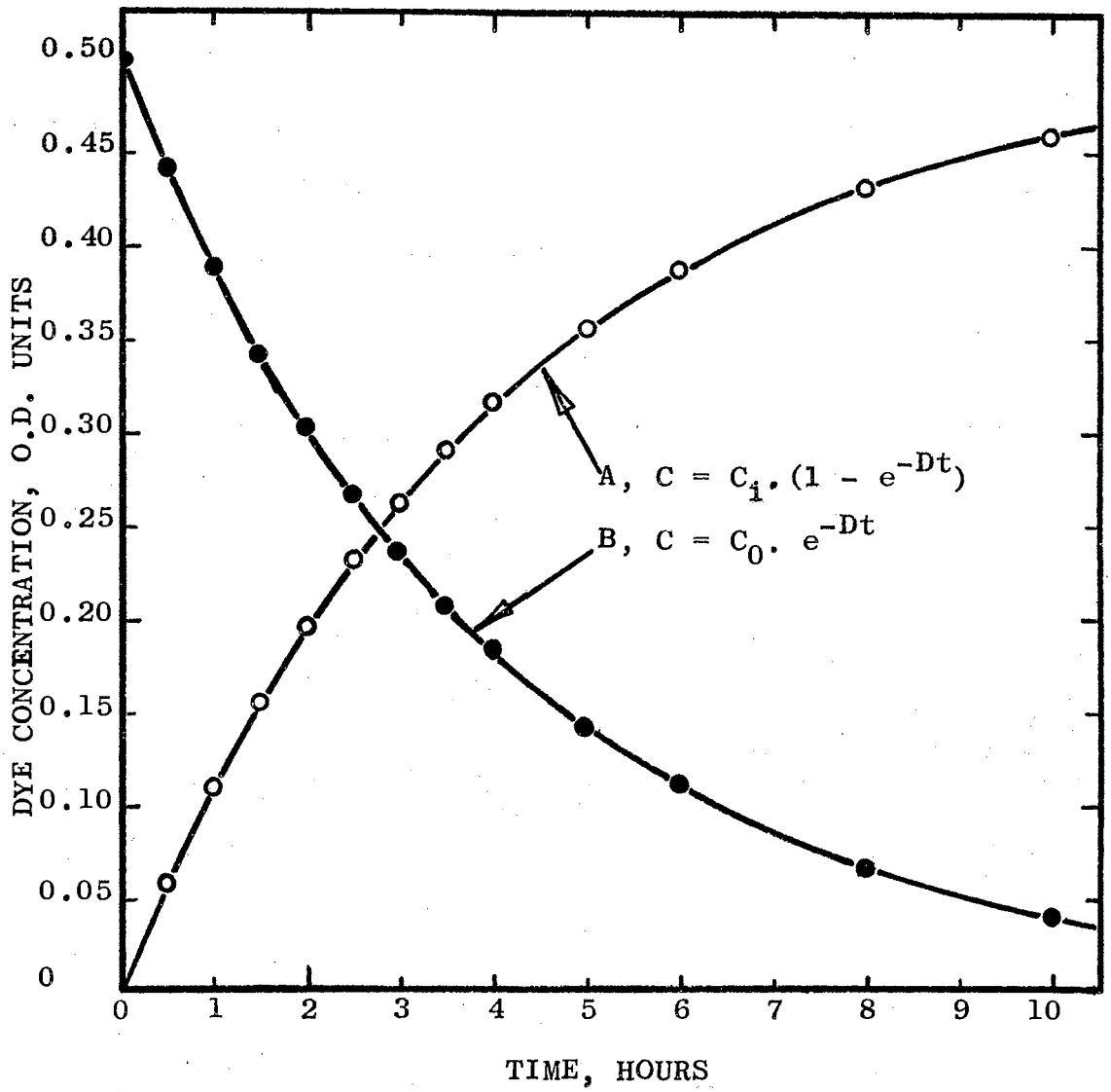

Figure 4. Flow Characteristics of a Continuous Flow Completely-Mixed System at D = 0.25 hr^{-1}; A - Increasing Concentration of Dye, and B - Decreasing Concentration of Dye.

will be discussed here. The steady state condition refers to the state in which the rate of growth of organisms in the reactor is equal to the rate at which they leave the reactor. Furthermore, the steady state condition refers not only to the microbial population but also to other system parameters. Also, under steady state conditions the output rate of cells is a constant and does not change with time. In other words, the output rate will be equal to the rate of growth and both will be constants. It can be seen from the above discussion that the organisms must be growing exponentially in a completely-mixed system when it is operated at steady state. Therefore, the growth of microorganisms in a completely-mixed system can be expressed mathematically as follows:

$$\frac{dx}{dt} = \mu x \tag{8}$$

where $\frac{dx}{dt}$ is the rate of change of cell concentration, μ is the specific growth rate, and x is the concentration of cells. For the research work reported here, the cell concentration is taken as dry weight of cells per unit volume. It is important to realize that there is a difference between cell weight per unit volume and cell numbers per unit volume. Growth of sludge on a weight per unit volume basis is used throughout the work, since it is impossible to assess the viable count accurately in a flocculent heterogeneous mass, and since viable count is not a parameter used in the operation of activated sludge.

The microorganisms involved in biological waste treatment are primarily bacteria, which multiply by binary fission. Therefore, the growth of bacteria can also be expressed by the equation

$$x = x_0 \, 2^n \tag{9}$$

where x is the concentration of organisms at any time t, x_0 is the initial concentration of organisms, and n is the number of generations which is equal to the time elapsed divided by the generation time or doubling time. James (14) has discussed the difference between mean generation time (G) and doubling time (T_d). According to his definitions, the doubling time refers to the time required for doubling in mass, and the mean generation time is the time required for doubling in number. The terms are herein used synonymously. Equation 9 can be written as

$$\log_e x = \log_e x_0 + n \log_e 2 \quad \text{where } n = \frac{t}{T_d} \text{ or } \frac{t}{G} \tag{10}$$

Differentiating Equation 10 with respect to t,

$$\frac{1}{x}\frac{dx}{dt} = \frac{\log_e 2}{T_d} \tag{11}$$

But $\frac{1}{x}\frac{dx}{dt}$ is equal to μ. Therefore

$$\mu = \frac{1}{x}\frac{dx}{dt} = \frac{\log_e 2}{T_d} = \frac{0.693}{T_d} \tag{12}$$

Equation 12 gives the relationship between specific growth rate and doubling time or generation time.

Relationship Between Specific Growth Rate and Substrate Concentration

The work of many researchers has revealed that the general relationships governing growth characteristics are similar whether the growth-limiting factor is the carbon, phosphorous, or nitrogen source. According to Monod (20), Novick and Szilard (7), and Dagley and Hinshelwood (24), the specific growth rate, μ, increases with increasing concentration of growth-limiting factor at very low concentrations. The rate of growth of a bacterial culture represents the overall velocity of all reactions by virtue of which cell substance is synthesized. When a single growth factor is present in limiting concentration, Monod (20) has shown that the specific growth rate (μ) is a function of the concentration of that limiting growth factor. This phenomenon can be represented by the differential equation

$$\frac{d\mu}{dS} = \alpha'(1-\mu) \tag{13}$$

where $\frac{d\mu}{dS}$ represents the differential of specific growth rate with respect to the concentration of limiting growth factor, and α' is the rate constant (25). Monod (20) proposed an approximate solution of the above equation as

$$\mu = \frac{\mu_m S}{k_s + S} \tag{14}$$

Where μ_m is the maximum value of μ, S is the concentration of the limiting growth factor, and k_s is the saturation

constant and is numerically equal to the substrate concentration at which $\mu = \frac{1}{2} \mu_m$. It is interesting to note that the above equation is similar to the Michaelis-Menten equation for enzyme reactions. Microbial growth can be considered as a result of a series of enzymatic reactions, and one might consider that the overall reaction rate is determined by the slowest reaction. Therefore, although the equation is empirical, one might loosely assign some theoretical significance to it.

Relationship Between Substrate Consumption and Cell Synthesis

Since the formation of each new cell may be considered to involve the consumption of a definite amount of substrate, growth can be expressed mathematically as

$$\frac{dx}{dt} = -Y \frac{dS}{dt} \tag{15}$$

where $\frac{dx}{dt}$ is the rate of change of cell concentration, $-\frac{dS}{dt}$ is the rate of substrate consumption, and Y is known as the yield coefficient and is assumed to be constant for a particular culture and substrate. Integrating the above equation, we get

$$Y(S_0 - S) = (x - x_0) \tag{16}$$

where x_0 and S_0 refer to initial concentrations of cells and substrate respectively. Thus, for a finite period of growth

$$Y = \frac{\text{weight of bacteria formed}}{\text{weight of substrate consumed}}$$

The parameters μ_m, k_s, and Y are considered to be the primary growth parameters, and can be readily determined from a batch culture (4). It is important to realize that two of the above parameters μ_m and k_s are applicable only to an exponentially growing system. The use of the above parameters in a completely-mixed system is discussed in the following section.

(c) Completely-Mixed Systems without Recirculation

Single-Stage Reactor

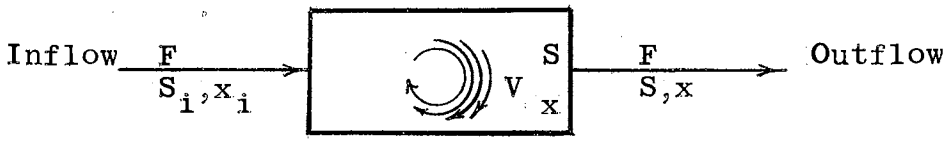

Figure 5. Schematic Representation of a Single Stage Completely-Mixed Reactor

The performance of the completely-mixed continuous flow process can be determined by material balance equations for both substrate and cell material.

Substrate balance:

$$\left[\begin{matrix}\text{Rate of change of sub-}\\ \text{strate in the reactor}\end{matrix}\right] = \left[\begin{matrix}\text{Rate of}\\ \text{inflow}\end{matrix}\right] - \left[\begin{matrix}\text{Rate of}\\ \text{outflow}\end{matrix}\right] - \left[\begin{matrix}\text{Rate of con-}\\ \text{sumption}\end{matrix}\right]$$

$$V \frac{dS}{dt} = F S_i - F S - \frac{\mu x}{Y} V$$

where S_i is the substrate concentration in the inflow.

$$\frac{dS}{dt} = \frac{F}{V} (S_i - S) - \frac{\mu x}{Y}$$

Since $\frac{F}{V}$ represents the dilution rate, D

$$\frac{dS}{dt} = D(S_i - S) - \frac{\mu x}{Y} \qquad (17)$$

The above equation gives the rate of change of substrate concentration in the reactor at any time.

Cell material balance:

$$\begin{bmatrix}\text{Rate of change of cell material in the reactor}\end{bmatrix} = \begin{bmatrix}\text{Rate of inflow}\end{bmatrix} + \begin{bmatrix}\text{Rate of growth}\end{bmatrix} - \begin{bmatrix}\text{Rate at which cells leave the reactor}\end{bmatrix}$$

$$V \frac{dx}{dt} = F x_i + \mu x V - Fx$$

where x_i is the concentration of cell material in the inflow.

$$\frac{dx}{dt} = Dx_i + \mu x - Dx \qquad (18)$$

When there are no cells in the inflow or when a sterile medium is pumped to the reactor, the term Dx_i reduces to zero, and Equation 18 becomes

$$\frac{dx}{dt} = \mu x - Dx \qquad (19)$$

Equation 19 gives the rate of change of cell material in the reactor at any time.

When the system is operating under steady state, neither the cell concentration or the substrate concentration changes with time. Therefore both $\frac{dx}{dt}$ and $\frac{dS}{dt}$ will be equal to zero.

$$\text{When } \frac{dx}{dt} = 0,\ \mu x - Dx = 0,\ \text{or } \mu = D = \frac{F}{V} \qquad (20)$$

Under steady state conditions the specific growth rate becomes equal to the dilution rate. In other words, biosynthetic process is controlled by the hydraulic flow rate in a completely-mixed reactor.

Also, Equation 19 reveals that steady state operations are possible only so long as the value of D is kept less than μ_m. When D is greater than μ_m, $\frac{dx}{dt}$ becomes negative and a complete washout of the culture will take place. The steady state values of substrate concentration ($\bar{S}$) and cell concentration ($\bar{x}$) can be determined from Equations 20 and 17. From Equations 20 and 14

$$\mu = D = \frac{\mu_m \bar{S}}{k_s + \bar{S}}$$

$$\frac{\bar{S} + k_s}{\mu_m \bar{S}} = \frac{1}{D}$$

$$\bar{S} = \frac{k_s D}{(\mu_m - D)} \qquad (21)$$

When $D \cong \mu_m$, $\bar{S}$ becomes equal to infinity; i.e., $\bar{S}$ will become equal to S_i and complete washout of the cells will occur. From Equation 17, when $\frac{dS}{dt} = 0$

$D(S_i - \bar{S}) = \frac{\mu \bar{x}}{Y}$; but $\mu = D$, and therefore

$$\bar{x} = Y (S_i - \bar{S}). \qquad (22)$$

The above treatment assumes: (a) cell material in the reactor is 100% viable, and (b) the substrate disappearance results only in the production of new cell material,

and (c) the organisms are growing always exponentially in the reactor.

Multi-Stage Reactor

In the interest of simplicity, let us consider two reactors of equal volume connected in series. The mixed liquor from reactor A flows into reactor B:

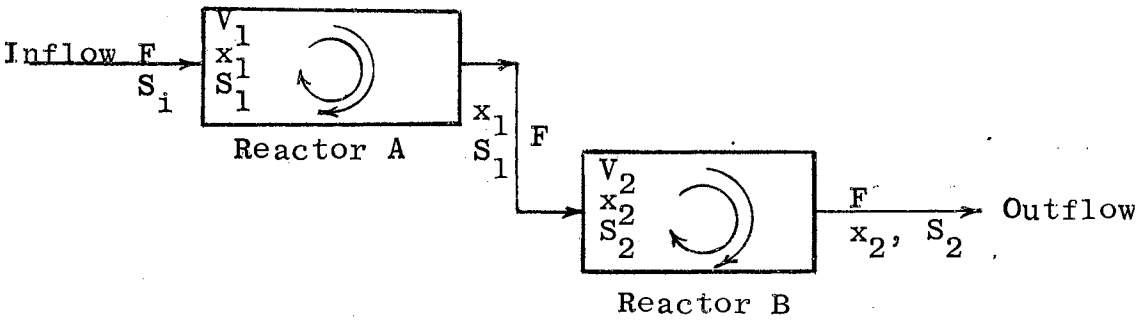

Figure 6. Schematic Representation of a Multi-Stage Completely-Mixed System

Reactor A will be similar in behavior to a single stage reactor, and the expressions for the steady state concentrations of substrate and cells will be identical to Equations 21 and 22. Let $\bar{x}_1$ and $\bar{S}_1$ be the steady state concentrations in reactor A. The material balance equations for reactor B can be written as follows:

Cell material balance:

$$\frac{dx_2}{dt} = D_2 \bar{x}_1 + \mu_2 x_2 - D_2 x_2$$

$$= D_2 (\bar{x}_1 - x_2) + \mu_2 x_2 \qquad (23)$$

During steady state,

$$\frac{dx_2}{dt} = 0$$

$$D_2 \ (\bar{x}_1 - \bar{x}_2) = - \mu_2 \ \bar{x}_2$$

$$\bar{x}_2 = \frac{D_2 \ \bar{x}_1}{(D_2 - \mu_2)} \qquad (24)$$

Substrate balance:

$$\frac{dS_2}{dt} = D_2 \ \bar{S}_1 - D_2 \ S_2 - \frac{\mu_2 \ x_2}{Y}$$

During steady state,

$$\frac{dS_2}{dt} = 0$$

$$D_2 \ \bar{S}_2 = D_2 \ \bar{S}_1 - \frac{\mu_2 \ \bar{x}_2}{Y}$$

$$\bar{S}_2 = \bar{S}_1 - \frac{\mu_2}{D_2 Y} \ \bar{x}_2 \qquad (25)$$

where S_2, x_2, D_2, μ_2, $\bar{S}_2$, and $\bar{x}_2$ refer to parameters of reactor B.

In a similar way the concentrations of substrate and solids for any number of reactors in series can be obtained.

(d) Completely-Mixed Systems with Recirculation

Only single stage systems will be discussed in this section, since theoretically the degree of treatment can be adjusted to any value with proper selection of a

recirculation factor. Let F be the flow rate of inflowing media, and αF be the rate of flow of recycle. The effluent from the reactor is allowed to settle in a settling tank or is centrifuged, and the concentrated sludge is recycled to the reactor at the rate of αF. For practical purposes the concentration of substrate in the recycle fluid can be neglected, as it contains mainly solids. Let the solids concentration in the recycle be equal to cx where c is termed a "concentration factor." The factor α is known as the "recirculation factor." The operation of a completely-mixed system with recirculation is shown in Figure 7. The performance of the system can be

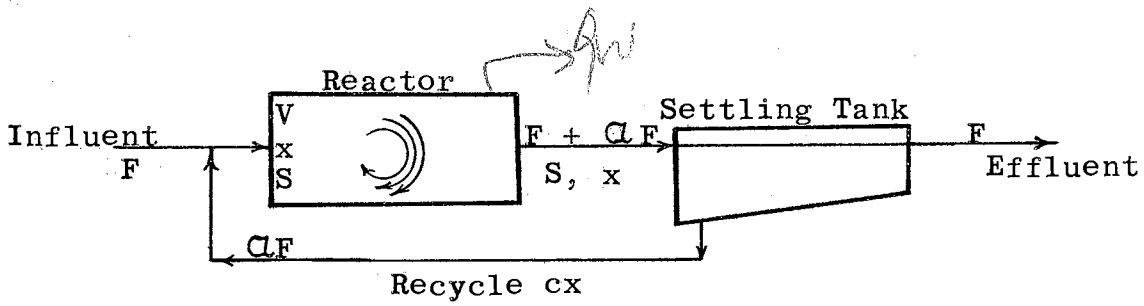

Figure 7. Schematic Representation of a Completely-Mixed Reactor with Recirculation

determined by materials balances for substrate and solids, as has been done for the system without recirculation.

Solids balance:

$$V \frac{dx}{dt} = F x_i + \alpha F cx + \mu x V - (1 + \alpha) F x \qquad (26)$$

Even though the actual dilution rate for a system with recirculation is $(1 + \alpha)F/V$, in order to avoid the com-

plication of introducing a new term, F/V itself will be considered as dilution rate, and the recirculation factor α is retained in the expression. From Equation 26, when $x_i = 0$,

$$\frac{dx}{dt} = D\ \alpha cx + \mu x - (1 + \alpha)Dx$$

During steady state $\frac{dx}{dt} = 0$, therefore

$$D\ \alpha cx + \mu x - (1 + \alpha)Dx = 0$$

or $$\mu = D\ (1 + \alpha - \alpha c) \qquad (27)$$

The specific growth rate is not equal to the dilution rate for a system with recirculation, but it is a function of D.

Substrate balance:

$$V\ \frac{dS}{dt} = F\ S_i + \alpha F\ S_R - (1 + \alpha)FS - V\ \frac{\mu x}{Y} \qquad (28)$$

where S_R is the concentration of substrate in the recycle. When there is no substrate in the recycle, the above equation can be written as

$$\frac{dS}{dt} = DS_i - (1 + \alpha)DS - \frac{\mu x}{Y}$$

When $\frac{dS}{dt} = 0$,

$$D\ \left[S_i - (1 + \alpha)\bar{S}\right] = \frac{\mu \bar{x}}{Y} \qquad (29)$$

or $$\bar{x} = \frac{DY\ \left[S_i - (1 + \alpha)\bar{S}\right]}{\mu}$$

But $\mu = D(1 + \alpha - \alpha c)$, and therefore

$$\bar{x} = \frac{Y\ \left[S_i - (1 + \alpha)\bar{S}\right]}{(1 + \alpha - \alpha c)} \qquad (30)$$

When the substrate concentration in the recycle is also taken into consideration, then the above equation is modified to

$$\bar{x} = \frac{Y(S_i - \bar{S})}{(1 + \alpha - \alpha c)} \tag{31}$$

where S_R has been taken as equal to S.

The steady state substrate concentration is calculated from the equations

$$\mu = D(1 + \alpha - \alpha c), \text{ and}$$

$$\mu = \mu_m \bar{S} \,/\, (k_s + \bar{S})$$

or $$\frac{\mu_m \bar{S}}{k_s + \bar{S}} = D(1 + \alpha - \alpha c)$$

from which it can be shown that

$$\bar{S} = \frac{k_s D(1 + \alpha - \alpha c)}{\mu_m - D(1 + \alpha - \alpha c)} \tag{32}$$

Substituting $D(1 + \alpha - \alpha c)$ equal to P', Equation 32 reduces to

$$\bar{S} = \frac{k_s P'}{(\mu_m - P')} \tag{33}$$

Also, it can be shown that the ratio of concentration of cells in the effluent to the concentration of cells in the aerator x_e/x can be expressed as follows:

$$\frac{x_e}{x} = (1 + \alpha - \alpha c) \tag{34}$$

The output rate of cells can be calculated from Equation 34 as:

Rate of output of cells = $D\ x_e$

$$= D\ (1 + \alpha - \alpha c)\ x \qquad (35)$$

In the absence of recirculation, the rate of output of cells is equal to Dx. By comparison of equations for straight-through and recycle systems it will be seen that addition of sludge recycle allows the system to be run at much faster flow rates, greatly increasing the output of cells. The amount of recycle sludge is restricted by the oxygen supply and other factors which impose limitations over and above that of the limiting nutrient.

In this section some of the basic kinetic theory and assumptions that have been used to predict the performance of continuous flow systems have been presented. They are, in general, those which have been considered by Herbert, et al. (4, 5). Many research workers have proposed modifications to the above approach, and these will be described in the literature review which follows.

CHAPTER III

LITERATURE REVIEW

The pioneer work of Utenkov in the Soviet Union, and Malek in Czechoslovakia has apparently influenced the study of continuous culture in other countries (11). Even before the advent of microbiology as a science, however, the ancient art of fermentation had progressed to the continuous production of vinegar in 1670, yeast manufacture in 1879, and sewage disposal in 1890 (11).

A revival of interest in the continuous flow process has taken place in the past fifteen years, not only because there is much scope for research in this field, but also because of the immediate usefulness of the process both in laboratories and in industries. The number of symposia held since 1958 and the number of reviews appearing in the literature attest to the continuing interest in the process. This has been aptly stated by Malek (26): "The continuous flow method of cultivation is becoming increasingly a necessary feature in experimental microbiology and represents a prerequisite in the development of industrial fermentations." The possibilities of applications of continuous flow processes are far from exhausted. In spite

of what has been demonstrated already, namely, that the continuous flow processes provide new possibilities for exact physiological, biochemical, and genetic studies by yielding microbial material of constant properties, which can be chosen at will, most experimental work is carried out using batch-grown cultures. This is due mostly to lack of basic knowledge and understanding about the process. In the following section are presented some of the developments which have taken place so far in the field of continuous flow processes.

A literature survey of the past work with continuous flow processes indicates that it can be conveniently separated into general groups: (1) equipment design and operation, (2) kinetics and mechanism, and (3) applications. The major portion of the research work has been in relation to fermentation processes even though a considerable amount of work has been done in other fields such as waste treatment, production of antibiotics, transformation of steroids, etc.

1. Equipment Design and Operation

Equipment design, development, and operation occupy a primary place of importance in any process research, and the continuous flow process is no exception. As early as 1944 Jordan and Jacobs (27) described the use of an automatic syringe mechanism for the continuous addition of nutrient solution. They maintained a constant volume in

the reactor by controlling the rate of evaporation so that it was equal to the rate of addition of nutrient solution. Evaporation was achieved by bubbling warm air through the system. This is similar to a closed system reactor, since no organisms leave the reactor. One disadvantage of this method of operation is that only water and certain volatile compounds escape the system by evaporation. This might result in the accumulation of other metabolic products, and toxic materials which may cause the failure of the system.

Myers and Clark (28) developed a device for continuous culture of photosynthetic organisms. A mixture of carbon dioxide and air was bubbled through the suspension. The carbon dioxide content in the mixture was 4.40 per cent. Illumination was provided by four tubular fluorescent or tungsten filament lamps. Algal cultures of uniform characteristics could be obtained using this procedure.

In 1948 Levin (26) obtained a patent for a culturing device. The process involved the formation of a circular lamella of liquid culture medium and inoculum. Novick and Szilard (6) described the working principles of a continuous culture apparatus, which they referred to as a "chemostat." The chemostat is an externally controlled system. The difference between externally and internally controlled systems was discussed previously. The mixing was accomplished by bubbling air through the system. The feed was supplied from a constant head reservoir. The

growth rate of the microbial population was controlled by a limiting nutrient concentration. They have shown that by controlling addition of the amino acid tryptophan, protein synthesis was ten times slower than when tryptophan was present in excess. At a tryptophan concentration of 10 µg/l, the growth rate was independent of tryptophan concentration (7).

Coe (30) has used a continuous flow process for the treatment of refinery wastes. The reactor used by Coe consisted of two chambers. In the primary chamber the biochemical transformation of organic materials to new cells took place, and the secondary chamber served as a settling unit. The settled sludge returned to the primary chamber by gravity. The process was primarily intended to reduce the effects of shock loading by slug dosage. It was not realized at that time that there could be a difference in the biochemical response between batch and continuous flow processes. Coe reported a removal efficiency of 90 to 95 per cent of BOD, and 90 to 95 per cent of phenol, with an aeration period of 9 to 12 hours and a suspended solids concentration of 4000 to 5000 mg/l.

Anderson (31) described the operation and principles of an "auxanometer," which is an internally controlled continuous culture system. An automatic recording of the growth rate was also described. The auxanometer is operated by a galvanometer at a predetermined bacterial den-

sity. When the bacterial density exceeds the predetermined value, it deflects the galvanometer and opens the valve, resulting in increased rate of addition of substrate. Due to the increased flow rate, the bacterial density drops and subsequently the rate of flow is also reduced. Thus the auxanometer controls the bacterial density in the reactor. There are certain requirments for the successful operation of this equipment, namely, (a) elimination of filming on those parts of the tube traversed by the light beam, and (b) adequate aeration without disturbance of the light beam. Anderson used a windshield wiper arrangement to prevent filming.

Hatfield and Strong (32) realized, while working with a toxic waste, that a batch-fed unit could tolerate higher BOD loadings per day when fed smaller amounts several times a day. They also realized that the continuously fed units would more nearly simulate actual plant practice than would those operated according to the usual fill-and-draw practice. They used a device similar to that of Coe (30) for the treatment of petrochemical, food processing, and paper mill wastes. BOD loadings of up to 164 lbs/day/1000 cu. ft. of aeration volume with 99.2 to 99.5 per cent efficiency in BOD removal for petrochemical wastes were reported. The efficiency of this process is comparable with the conventional process, even though the loading is much higher than in the conventional process.

Elsworth, et al. (33) described the design and construction of a continuous culture apparatus with a two-liter vessel. Aeration was achieved by means of a mechanical stirrer and compressed air. Devices were incorporated for automatic temperature and pH control. Foaming was controlled by adding antifoam through a manually controlled valve. The apparatus could be operated up to 1000 hours without problems of contamination.

In 1958, McKinney, et al. (34) realized that the fundamental problem in the conventional activated sludge process is the continually oscillating population due to plug flow operation. They designed a completely-mixed continous flow process for treating cotton textile plant wastes. The bleachery wastes had a BOD of 950 mg/l, and pH of 11.0. The pH of the raw waste was adjusted to between 9 and 10 before use in the pilot plant. They found that BOD loadings up to 60 lbs/day/1000 cu. ft. of aeration volume could be applied successfully even without preneutralization. The system was operated as a closed system, and by hydraulic model studies it was found that the position of the baffle in the settling section will determine the degree of settling. They also concluded that the cost of constructing such a plant was one-third that of the conventional plant, and that the plant is fully automatic.

Busch (35) emphasized the importance of simulating actual plant conditions in laboratory bench scale experiments. Bench scale experimental data can yield valid information only if the process variables are controlled. Bench scale units should have sufficiently large volumes of culture to permit withdrawal of samples without appreciably affecting the food-solids balance. The sample size required for valid analysis should represent not more than one per cent of the mixed liquor. Operation of one such unit was described by Busch (35).

Busch and Myrick (36) compared the operations of batch and continuous flow systems. The units were operated as closed systems without sludge wasting. They concluded that neither the batch nor the continuously operated system could function indefinitely without sludge wasting. Differences were observed in the batch and continuous systems in both settling and solids characteristics such as volatile content and sludge volume index. No food-population equilibrium was reached even after 103 days of operation.

Solomons (37) discussed the factors affecting aeration and agitation in continuous cultures. Oxygen transfer rate is a function of turbulence and the chemical composition of the fluid. The chemical composition can affect oxygen transfer rate in two ways besides the surface tension effect. The solutes present in the mixed liquor reduce the solubility of oxygen and the Newtonian

viscosity of the fluid. Solomons also reported that the mycelium viscosity is the largest single factor in reducing the oxygen transfer rate. The mycelium viscosity refers to the viscosity of a suspension of filamentous organisms and particularly of fungal growth. While a bacterial culture may have more oxygen demand, the culture itself usually remains Newtonian and its oxygen transfer rate remains nearly constant. When filamentous molds grow, they produce a broth that is non-Newtonian.

Fuld, et al. (38) discussed the importance of the study of the dynamic nature of microbial populations in a continuous flow process. The dynamic nature refers to the rate and manner in which the system responds to changes in the ambient conditions. The use of a sinusoidal forcing function for pH was described. The pH was varied between 5 and 6 by an automatic device, and the amplitude and frequency of the sinusoidal wave for pH were determined. The responses to the variation in pH were determined by measuring bacterial density and substrate concentration.

Quinn (39) described a continuous culture apparatus for the growth of rumen microorganisms in a chemically defined medium. Oxidation-reduction potential and pH were controlled by a Beckman automatic titrator using standard glass, calomel, and platinum electrodes. Agitation of the culture by gas diffusion was coupled with mechanical stir-

ring to keep the culture homogeneously mixed. In a fermentation review article it has been shown that the degree of mixing depends not only on the agitation power per unit volume, but also on the scale of the system (40). Dawson's description of a unique continuous flow apparatus based on the cyclone column principle was also given in the review (40). One of its reported advantages was that it could be used for propagation of filamentous cellular systems.

Foster and Litchfield (41) have described a continuous culture apparatus for the microbial utilization of hydrogen produced by the electrolysis of water in a closed cycle space system. The surplus hydrogen produced by electrolysis may be utilized together with waste CO_2, part of the oxygen, and waste urea by bacteria of the genus Hydrogenomonas to produce cellular protein which might be used as a source of food. This system appears to be promising from the standpoints of energy, weight requirements, and ability to operate in space vehicles.

Holmstrom and Heden (42) developed a continuous culture apparatus made from six small (80 to 800 ml) glass units with independent pH, aeration, and foam control. Exchangeable attachments made it possible to run the units both batchwise and continuously, and to connect up the units in various fashions. The equipment can be adapted to the cultivation of aerobic or anaerobic organisms and pathogenic or non-pathogenic organisms. It also makes

possible the use of a recirculating gas for the optimum utilization of volatile substrates and intermediates.

Tempest (43) discussed the need for an adequate supply of microorganisms in microbiological and biochemical research, and the suitability of continuous culture for greater output and quality control of the culture. He developed an 8-liter continuous flow apparatus with automatic control of temperature, pH, and foam, medium and air flow control, and a system for handling large volumes of sterile media. Fiechter (44) discussed the importance of satisfactorily functioning equipment for the reliable maintenance of steady state conditions. Equipment malfunction could be the most important reason for instability in the continuous culture operation. He described an apparatus which is specifically designed for high reliability and simplicity of service. In order to ensure the elimination of contamination he introduced sterilizable pH electrodes, and a membrane enclosure in the apparatus.

2. Theory and Kinetics of Continuous Flow Process

Thus far a review of the developments in equipment design and operation has been presented. From the foregoing presentation it can be seen that there are different nomenclatures for the reactor of the continuous flow process. Names such as "turbi-stat," "bactogen," "breeder," "microgenerator," and "symbiocon" have also been used in the literature to describe the reactor of a continuous

flow system (14, 45, 19). Similarly, different symbols have been employed by different researchers to describe the growth parameters. In this dissertation the symbols used by Herbert (4) have been adopted.

Kehr (22) attempted to analyze the detention time of liquids being mixed continuously. Tests on the detention time were made with uranin and salt solutions in rectangular tanks. He also worked out mixing patterns in a series of tanks, and discussed the application of the equations to activated sludge processes and sludge digestion. The equations are identical to Equations 5 and 6 presented in the previous chapter.

Sawyer and Rohlich (46) were probably the first to realize that the conventional activated sludge process resembles a completely-mixed continuous flow process. They operated two identical units, one in the conventional manner, and the other with stage addition of return sludge. Their results indicated that it made little difference where the return sludge was added in the aeration tank. The same concentration of suspended solids was observed both at the inlet end and at the outlet end of the reactor, regardless of the position of the return sludge addition. Uniformity was observed not only in sludge concentration, but also in the value of oxygen utilization and the distribution of nitrate throughout the length of the aeration tank. This probably led Garrett and Sawyer (18) to

propose the kinetic theory of the conventional activated sludge process based on the completely-mixed system. Busch (35) found that the bench scale experimental data often do not agree with the pilot plant or full scale results. He attributed this difference to the differences in process environment.

Deindoerfer and West (47) have shown the importance of rheological properties of fermentation broths in the design and scale of fermentation processes. Broths of streptomycin and penicillin have been shown to possess non-Newtonian behavior. The behavior of fermentation broths depends upon the type of organism, their size and shape, and their concentration. When such fluids are encountered in a process, it is important to modify the expressions for operations such as fluid flow in pipes, agitation, mass transfer, heat transfer, and bubble and particle dynamics. Solomons (37) also referred to the non-Newtonian behavior of a suspension of filamentous molds and their effects on oxygen transfer rate.

Grieves, et al. (48) have derived mathematical expressions to describe the flow regime in a reactor under different mixing conditions. They have used a "volume apportionment method" to divide the system into different mixing patterns. Relations which consider an effective volume of complete mixing, stagnant zones, and shortcircuit and plug flow conditions were derived. A completely-

mixed system is identified by the homogeneous conditions in the reactor, and by the characteristics of the effluent and mixed liquor. Under ideal conditions the effluent and mixed liquor should be identical in all respects. Milbury, et al. (49) have presented experimental procedures for evaluating the different constants of the volume apportionment method. Studies were conducted in rectangular tanks holding nine liters of liquid. The tracer substances used were sodium chloride and skim milk solutions. Different mixing patterns were induced by introducing baffles at different locations in the tank. Mixing was accomplished by air diffusion. In a succeeding article by the same authors (50) they have compared the volume apportionment method with the "axial dispersion method" for the prediction of mixing patterns. The axial dispersion method assumes that plug flow occurs in the aeration tank. Longitudinal mixing is described by assuming random fluctuations occurring in an axial direction. They have concluded that the volume apportionment method is more suitable for biological reaction, and the axial dispersion method is limited to describing mixing conditions in a single reaction vessel. A more detailed analysis of the axial dispersion method is given by Dawkins (51). Sack and Schulze (52) have used complete mixing theory to describe processes in which the suspended solids concentration in the reactor is always greater than that in the effluent. The ratio of

effluent solids concentration to the reactor solids concentration varied between 0.50 and 1.0. Even though this ratio represents an incomplete mixing condition, they have used a "retention factor" (L) to correct for this, and modified the basic equations by including the retention factor.

Substrate Consumption and Growth

As early as 1938 Dagley and Hinshelwood (24) showed that the exhaustion of nutrients rather than the accumulation of toxic products is the factor limiting growth. When Lactis aerogenes was grown in a synthetic glucose-phosphate-ammonium sulfate medium, the final concentration reached was proportional to the concentration of nutrients. Hinshelwood (53) has shown the relationship between the inoculum size and specific growth rate determination, and recommended a small inoculum size to reduce the error in the measurement of specific growth rate. The theory involved was that when the inoculum is large, the ratio between the dead cells and living cells will be increased and the specific growth rate measured will be only an apparent value. The relationship between apparent specific growth rate and number of dead cells in the inoculum has been shown to be

$$\frac{x_d}{x} = 1 - \frac{\mu_{app}}{\mu} \qquad (36)$$

Where x_d is the concentration of nonviable population in the inoculum, μ_{app} is the observed specific growth rate, μ is the value of specific growth rate when 100 per cent viability is assumed in the inoculum, and x is the concentration of microbial population at any time. Hinshelwood has also discussed the relationship between growth rate and substrate concentration, and the methods of determining specific growth rate.

Monod (20) has shown that bacterial density and bacterial numbers will be proportional in a growing system if the average size of the cells does not change, provided the system has 100 per cent viability. He has also stated that indicators such as nitrogen disappearance, estimation of metabolic activity, oxygen consumption, acid production, and optical density could be used to determine rate of growth. Monod has further shown that the relationship between substrate consumption and protoplasmic accumulation is a constant for a given substrate under similar conditions. This relationship is known as the "yield coefficient" (Y). The relationship between substrate concentration and specific growth rate proposed by Monod has been presented in the previous chapter. Van Niel (54) has discussed the various aspects of growth in a batch culture with particular reference to the theories of space limitation of growth, and limitation due to insufficient oxygen supply. He has concluded that growth curves of

microorganisms are as a rule accurately reproducible if the environmental conditions are kept the same.

Adams and Hungate (55) have conducted experiments with yeasts, utilizing various substrates of economic importance such as pear, apple, and cherry juice. From the batch growth curve they have calculated the value of growth rate, $\frac{dx}{dt}$, and specific growth rate (μ). The reciprocal of the specific growth rate has been referred to as "cycle time," which is equivalent to mean residence time in continuous culture. From their experiments they have concluded that by operating a continuous flow process at the cycle time determined from a growth curve, a steady state condition as described by the growth curve can be achieved. They have also concluded that the physiological states of the cultures are identical in both the batch and continuous culture.

Garrett and Sawyer (18) have proposed methods to determine specific growth rate values from oxygen uptake or from substrate disappearance. They found a maximum value for μ in the range of 0.21 per hour at 20°C., and 0.26 per hour at 30°C. The experiments were conducted with initial substrate concentrations of 400 to 500 mg/l 5-day BOD at 20°C. Glucose and peptone were used as substrates. They found, experimentally, a straight line relationship between the BOD remaining and dilution rate under steady state conditions.

$$\bar{L} = 363\ \mu + 17 \qquad (37)$$

They assumed the specific growth rate to be equal to the dilution rate (D). The specific growth rate varies according to Equation 37 up to a critical value of BOD, and then remains constant.

Golle (56) has discussed the advantages of continuous culture for the production of microorganisms. The specific growth rate was obtained from batch experiments during an exponential phase of growth. He considered the exponential growth rate as a constant, and obtained material balance equations for the continuous flow process.

Finn and Wilson (57) have studied the growth of Pseudomonas fluorescens, Brevibacterium linens, and Saccharomyces carlsbergensis in batch units, and in chemostats. They found that both Ps. fluorescens and B. linens exhibit an exponential growth phase. Continuous flow was started when the growth had reached a desired level of population. It was found that the steady state population in the chemostat was not uniquely determined by the dilution rate (D), but that the level of population depended upon the population level at which the continuous flow operation was started. The theoretical explanation which they offered for this behavior was that the specific growth rate remains constant during the exponential phase even though the population density changes. Therefore, any population density within the exponential phase can be maintained at steady

state in a chemostat provided that level of population is maintained before the start, and the flow rate through the reactor is kept at a value such that the dilution rate (D) is equal to the specific growth rate (μ). With respect to the cultivation of Sacch. carlsbergensis, it was found that the growth does not proceed exponentially for very long. This finding was thought to be the result of a drop in pH when the sugar was converted to acid. The fluctuations in pH were 90 degrees out of phase with the fluctuations in solids concentration, due to the lag in response of the culture.

Gaden (58) has discussed the applicability of various process relationships to biological processes. The first consideration of any process is its stoichiometric relationships. Except in a few fermentation processes (ethanol and gluconic acid) the stoichiometric relationships are unknown; therefore the product formed per unit time is used as a measure of efficiency. The interpretation of energy relationships in biological processes is also complex. Finally, he has discussed the advantages of continuous processes over batch type. These have already been presented in the previous chapter.

Maxon (8) has presented a review of the continuous fermentation process in which the specific growth rate is considered as a function of substrate concentration. This relationship is the same as those proposed by Monod,

Novick and Szilard, and others. Maxon has developed equations for the introduction of undesirable organisms and mutants into the continuous culture reactor. He has also described the two-phase continuous flow systems in which the biochemical transformation takes place in liquid and solid phases. An example of such a process would be vinegar or acetic acid fermentation. Here the fermentor consists of beechwood shavings upon which the Aerobacter, which is responsible for the oxidative conversion of ethanol to acetic acid, is fixed.

Powell (59) has derived certain relationships between specific growth rate (μ), generation time distribution, and age distribution in growing cultures. The generation time distribution curve has been shown to be close to Gaussian, and the effect of inheritance on generation time is probably negligible. He has also shown that the general equations that have been used for growth in continuous culture are usually false, i.e., Equations 11 and 20 presented in the previous chapter. It has also been shown that the true relations differ from Equations 11 and 20 only slightly.

Herbert, et al. (5) have dealt with theoretical aspects of continuous culture systems which allow quantitative prediction of steady state concentrations of substrate and cells. Experimental results obtained with Aerobactor cloacae in a chemically defined medium confirm

the theoretical equations developed. These equations do not differ basically from those presented in Chapter II.

Sawyer (60) has presented the relationship between growth and substrate consumption. He has reported a yield coefficient of 50 to 60 per cent of the dry weight of organic food material consumed. For glucose, 44 to 64 per cent can be expected. This agrees with results reported by Helmers, et al. (61) for cotton kiering, rag-rope kiering, and brewery wastes. They have reported a yield coefficient of 53 to 60 per cent. Gellman and Heukelekian (62) have reported on studies of sludge growth during biological purification of jute cook liquor, yeast waste, gum waste, and board mill white water. A summary of their data shows a yield of 0.50. Porges, et al. (63) reported a yield coefficient of 0.57 to 0.63 in the treatment of skim milk waste by a continuous flow process. This figure agrees reasonably well with others reported.

Rao and Gaudy (16) have reported from their experiments with activated sludge that the yield coefficient varied between 0.48 and 0.82. They concluded that the variation in yield might be due to predominance or selection. Servizi and Bogan (64) related the yield coefficient to moles of ATP produced in the oxidation of a particular substrate. They have also related ATP produced to free energy of oxidation (ΔF^{o}_{ox}) which in turn was related to the COD of the substrate.

$$Y = k_4 N_{ATP} \tag{38}$$

where k_4 is a constant of proportionality in units of grams per mole of ATP, and N_{ATP} is the moles of ATP made available during metabolism of one mole of substrate. When the metabolic pathways are known, the ATP yields can be calculated. If ATP yield is related to the free energy released, then

$$N_{ATP} = -k_5 (\Delta F^o_{ox}) \tag{39}$$

and $$Y = -k_4 k_5 (\Delta F^o_{ox}) \tag{40}$$

If ΔF^o_{ox} is proportional to the moles of oxygen that are required for theoretical oxidation of one mole of substrate, that is to say

$$\Delta F^o_{ox} = - k_6 Z$$

Then $$Y = k_4 k_5 k_6 Z \tag{41}$$

Servizi and Bogan (64) found that the ratio of free energy to yield was different for different groups of substrate. Carbohydrates and Krebs cycle intermediates gave a value of 0.166 g/kcal for k_4k_5. Aromatic and aliphatic acids gave a value of 0.13 g/kcal. for k_4k_5. They also concluded that the fraction of substrate oxidized to CO_2 and H_2O is proportional to its COD, carbon content, and chemical nature, and these are related as follows:

$$f_o = 1 - k_7 \frac{COD}{C_2} \tag{42}$$

Where f_o is the fraction of substrate oxidized, k_7 is a constant, and C_2 is carbon content of the substrate expressed as a percentage by weight. While the above method seems to be interesting, it does not take into consideration that a compound can have COD whether it is biodegradable or not. Another point to be considered is that the ATP yield is different for different pathways, and a single organism can metabolize a compound in more than one pathway. Therefore the yield cannot be related to COD directly unless the biochemical pathways of the compounds are known under the experimental conditions.

Eckenfelder and Weston (65) have shown that the sludge growth and BOD removal are associated in the following manner:

$$\text{Biological volatile solids produced} = a(\text{BOD removed}) - b(\text{MLVSS}) \quad (43)$$

where a and b are constants and MLVSS represents mixed liquor volatile suspended solids concentration. From batch culture experiments they have shown that the maximum rate of substrate removal is associated with the exponential growth phase, and they have considered the exponential growth rate as maximum. The value of μ_m was taken as equal to 0.30 hr^{-1} at 30°C. and 0.08 hr^{-1} at 20°C.

Wuhrmann (66) has discussed the dissimilarities in the specific purification performance of domestic wastes. Specific purification rate refers to the rate of BOD re-

moval per unit weight of cells. According to Wuhrmann, differences in the purification capacity are due to the differences in absolute number of actively metabolizing cells per unit weight of sludge. He has proposed a method for determining the active portion of the sludge mass. The method involves the measurement of the oxygen consumption rate of a washed sludge suspension. This method will be referred to later in this work. Even though this method might give different results due to the differences in storage products, it appears to give a satisfactory measure of the active portion of the sludge mass.

Pirt (10) has developed equations to relate oxygen uptake rate and growth rate of microorganisms. Since this method is employed in the present research work, it is presented here in detail. Let p moles of oxygen be consumed for each mole of limiting substrate utilized. Then, if o is the concentration of dissolved oxygen at any time t, the rate of oxygen utilization can be written as:

$$-\frac{do}{dt} = -p\frac{dS}{dt} \tag{44}$$

The rate of substrate consumption is related to growth according to Equation 14

$$\frac{dx}{dt} = -Y\frac{dS}{dt} = \mu x \tag{14}$$

Also,

$$Y(S_0 - S) = (x - x_0) \tag{15}$$

or $x = Y(S_0 - S)$ when $x_0 << x$.

Therefore

$$-\frac{do}{dt} = \frac{p}{Y}\frac{dx}{dt} = \frac{p}{Y}\ \mu x \qquad (45)$$

and

$$-\frac{do}{dt} = p\ \mu\ (S_0 - S) \qquad (46)$$

For a continuous flow system operating at steady state, μ can be replaced by D, and when $S << S_0$, Equation 46 becomes

$$-\frac{do}{dt} \approx p\ D\ S_0 \qquad (47)$$

Equation 47 suggests that the rate of oxygen uptake is a function of dilution rate (D) and the substrate concentration in the inflow medium. Pirt has also related "specific catabolism rate" (k_c) to specific growth rate (μ). Specific catabolism rate refers to the rate of substrate catabolism per unit weight of organism.

$$-\frac{dS}{dt} = k_c x \qquad (48)$$

From Equation 14, $x = -\frac{Y}{\mu}\frac{dS}{dt}$

Therefore, from Equations 14 and 48

$$k_c = \frac{\mu}{Y} \qquad (49)$$

Pirt has verified the application of the above equations in growth studies with Aerobacter cloacae strain 8197. At low dilution rates no volatile acids were produced. When the dilution rate was numerically equal to 0.85 μ_m, volatile acids were formed. Also, the growth rate constants of batch cultures varied from one culture

to another and were generally lower than the maximum growth rate constant obtained in continuous culture.

Garrett (67) has related specific growth rate to sludge age. Since μ is equal to D under steady state conditions, it can be shown that the reciprocal of μ or D is equal to the pounds of volatile suspended solids in the reactor divided by the pounds of volatile suspended solids wasted per day, which is a measure of sludge age. Therefore, if the specific growth rate is controlled directly, the pounds of BOD removed per day per pound of volatile solids in the aeration tank, the sludge age and the effluent BOD can be controlled. This suggests that the growth rate can be used as a direct measure of control in the operation of waste treatment plants, and analytical determinations such as BOD and suspended solids could be eliminated.

Moser (19) realized that each unit of reactant and reaction product in a completely-mixed continuous flow reactor has an equal probability, $p'(\Delta t)$, of leaving the reactor within a certain time interval Δt. Although the probability is constant, some of the organisms remain in the culture vessel for a considerably longer time and some for a considerably shorter time than the mean residence time, $\bar{t}$. He has also pointed out that the growth of nonfilamentous bacteria in a continuous growth apparatus is governed by four different rate processes:

(a) rate of formation of cells by binary fission

(b) rate of production of nonviable cells

(c) rate of removal or washout of bacterial suspension, and

(d) rate of assimilation of limiting nutrients.

Of the above four different processes, three have already been discussed in detail. The rate of production of nonviable cells is used here to denote the production of cells that fail to reproduce further offspring, but remain intact in the culture. This rate is a function of the concentration of viable population (x_v).

$$\frac{dx_{nv}}{dt} = - \frac{dx_v}{dt} = v\ x_v \qquad (50)$$

where x_{nv} is the concentration of nonviable cells. Equation 50 indicates that the rate of increase of nonviable cells is accompanied by a concurrent decrease in viable population, which is logical. The rate constant v is called "nonviable constant" or "specific mortality rate." The general equation for exponential growth $\frac{dx}{dt} = \mu x$ has to be modified to consider the production of nonviable cells.

Moser has also derived expressions for the growth of mixed populations having different generation times in the continuous flow reactor. If we consider j types of organisms growing simultaneously having specific growth rates μ_j and corresponding specific mortality rates v_j, then

the rate of increase of each type of organism can be written as follows:

$$\frac{dx_1}{dt} = \mu_1 x_1 - v_1 x_1 - D x_1$$

$$\frac{dx_2}{dt} = \mu_2 x_2 - v_2 x_2 - D x_2$$

In general

$$\frac{dx_z}{dt} = \mu_z x_z - v_z x_z - D x_z \qquad (51)$$

Similarly, equations can be written for substrate catabolism rate. From Equation 48

$$-\frac{dS}{dt} = k_c x_v \qquad (52)$$

Since only viable cells are responsible for substrate assimilation, only those are considered in the above equation. It is also assumed that there are no resting cells in a continuous flow reactor in which the organisms are multiplying exponentially. The nonviable fraction is 100 per cent dead cells, and therefore they do not contribute to substrate removal. Rewriting the above equation for a mixed culture with substitution for k_c from Equation 49

$$-\frac{dS}{dt} = \sum_{j=1}^{j=z} \frac{\mu_j}{Y_j} x_{v_j} \qquad (53)$$

One important consideration in the continuous cultivation of mixed cultures is that the value of μ_{jm} should be greater than the operating dilution rate. If any μ_{jm} value is less than D, the washout of that species will

occur. A pure culture containing mutants also follows the same kinetics as that of a culture of different organisms.

Moser has also proposed a modified expression for the specific growth rate, which is similar to that proposed by Monod, except that the exponent of limiting nutrient concentration is not unity.

$$\mu = \mu_{max} \left[\frac{1}{1 + k_s S^{-\lambda}} \right] \qquad (54)$$

When λ is equal to unity, the equation reduces to Monod's. Powell (68) has also discussed the kinetics of growth of mutants and contaminants in continuous culture. He concluded that the contaminants and mutants can grow successfully only when their maximum growth rate (μ_m) and saturation constant stand in a constant relation to that of the native organisms and to the rate of flow through the culture vessel.

Stewart, et al. (21) have applied the kinetic theory of the continuous flow process to verify, experimentally, the kinetics of anaerobic processes. The rate of utilization of substrate is expressed algebraically in terms of volatile suspended solids accumulation in the following manner:

$$\text{Rate of substrate utilization as VSS} = (s + u + r)\, a' s_r \qquad (55)$$

where

s = fraction of removed substrate that is synthesized to new cell material or that disappears from the system due to endogenous respiration

u = fraction of removed substrate that is stored in the cell as part of net growth

r = fraction of removed substrate that is respired for the production of energy

a' = conversion factor between COD and VSS

s_r = rate of substrate utilization per unit weight of organisms.

Since the differential determination of synthesis and storage is impracticable, the storage term, u, can be omitted from Equation 55. Therefore, Equation 55 reduces to $(s + r)\ a'\ s_r$. Further, they have assumed the fraction of substrate removed for synthesis to be a constant fraction of total substrate removal. Therefore

$$s\ a'\ s_r = c'\ s_r \tag{56}$$

where c' is an assumed constant that combines a' and s. They have also assumed that the substrate consumption for synthesis is the sum of substrate consumed for endogenous respiration and net synthesis or net accumulation of VSS. Therefore, Equation 56 can be written as follows:

$$c'\ s_r = \begin{matrix}\text{specific endogenous} \\ \text{respiration rate}\end{matrix} + \begin{matrix}\text{specific growth} \\ \text{rate}\end{matrix} \tag{57}$$

$$c'\ s_r = E + \mu \tag{58}$$

During steady state operation of completely-mixed continuous flow process $\mu = D = \frac{1}{\bar{t}}$ and Equation 58 can be written as follows:

$$c' s_r = E + \frac{1}{\bar{t}} \tag{59}$$

or $$\frac{1}{\bar{t}} = c' s_r - E \tag{60}$$

By plotting a curve $\frac{1}{\bar{t}}$ versus s_r, a straight line relationship was observed from which the constants c' and E were evaluated. They have also related s_r to the substrate concentration by an equation similar to that for specific growth rate (μ)

$$s_r = \frac{s_{rm} S}{U + S} \tag{61}$$

where s_r can be termed specific substrate assimilation rate and S is the concentration of substrate, s_{rm} is the maximum value of s_r, and U is the substrate concentration at which $s_r = \frac{1}{2} s_{rm}$. It is important to note that s_r is the same as k_c used in Equation 48, where k_c has been referred to as catabolism rate. Stewart, et al. have verified the Equations 60 and 61 from anaerobic fermentation experiments.

Deindoerfer and Humphrey (17) have presented stoichiometric equations for conditions involving growth and product formation, such as production of ethanol, etc.

$$- \alpha_1 \frac{dS}{dt} = \beta \frac{dx}{dt} + \gamma \frac{dP}{dt} \tag{62}$$

where α_1, β , and γ are constants and the ratio $\alpha_1 : \beta : \gamma$ is also a constant. They have also presented graphical determinations of steady state concentrations of process parameters, both for single and multi-stage units. Leudking and Piret (12) have used the basic differential equations of the continuous flow process obtained by material balance to predict the concentrations of substrate and suspended solids for transient conditions. While solving the differential equations they have assumed the value of specific growth rate as a constant. Transient conditions will be introduced in a continuous flow system when there is a change in dilution rate or when there is a change in the inflow substrate concentration. These can also be referred to as "hydraulic shock loading," and "quantitative shock loading," respectively. Leudking and Piret (69) have presented another form of stoichiometric equation for reactions involving products other than microorganisms.

$$\frac{dP}{dt} = \alpha_2 \frac{dx}{dt} + \beta_2(x) \qquad (63)$$

The constants α_2 and β_2 have been found to be a function of pH in the study of lactic acid fermentation.

Northam (70) has developed equations to predict the course of changes in population and substrate concentration from the time of inoculation to the time a steady state is attained in a continuous flow process. These

equations are not presented here, since they apply only to the process of establishment of steady state conditions.

Contois (71) felt that the specific growth rate is not only a function of substrate concentration, but also a function of suspended solids concentration. He has proposed a modified expression for specific growth rate as follows:

$$\mu = \frac{\mu_m S}{Bx + S} \tag{64}$$

where μ_m and B are growth parameters. Contois verified the applicability of the above equation by growing A. aerogenes in batch and continuous cultures. A nonlinear decrease in the value of μ was observed with increasing values of population density.

Borzani, et al. (72) considered the rate of substrate utilization as a function of cell concentration and substrate concentration.

$$-\frac{dS}{dt} = F(x,S) \tag{65}$$

They proposed an expression for substrate utilization

$$-\frac{dS}{dt} = K \times S^q \tag{66}$$

Experimentally they found when $q = 0.50$, the best fit for experimental data occurred. It is interesting to note when $q = 0$, Equation 66 reduces to Equation 52 proposed by Moser (19).

Ware (73) has discussed the continuous flow process as applied to waste treatment. According to Ware, the sewage treatment plant is a "natural habitat" for a wide variety of organisms which are directly or indirectly responsible for the overall process of purification. Therefore it is necessary to maintain all types of organisms in the reactor. When this is required, the flow through the reactor should be maintained at such a level that even the slowest-growing organisms are not washed-out of the reactor. It has been shown previously that the value of D should be lower than μ_m in order to prevent a washout of the culture.

Deindoerfer (25) has reviewed the various approaches to determining specific growth rate. In addition to Equations 14 and 54, he has also considered the one proposed by Teissier, which is an accurate solution of Equation 13.

$$\mu = \mu_m \left[1 - e^{-S/k_s}\right] \qquad (67)$$

Deindoerfer concluded that Monod's expression can be used when there is only one growth-limiting factor present in the medium. Dawson (74) conducted experiments with Saccharomyces rouxii in continuous culture, and showed that the specific growth rate increases with increasing oxygen supply. Also, a selection between "fully aerobic," "partially aerobic," and "microaerophilic" organisms developed with differences in air supply. From experiments conducted with a constant amount of glucose and

different amounts of nitrogen he has shown that yield calculated with respect to glucose was constant for all nitrogen levels. But the yield calculated from nitrogen uptake increased with increasing nitrogen concentration. This is probably due to glucose being channelled to storage products at low nitrogen levels.

Maxon (75) conducted studies with continuous culture, and has shown that yield coefficient is not a constant at all dilution rates. He observed low yields at low dilution rates; the yield increased with dilution rate up to a certain value, and then decreased again. He has reasoned that the lower yields at low dilution rates are due to increased rate of endogenous respiration, but Stewart, et al. (21) have shown the rate of endogenous respiration is a constant and does not change in the presence or absence of exogenous substrate. Therefore, Maxon's interpretation is probably subject to doubt. The variation in yield with changing dilution rate might possibly be due to variations in the amount of storage products within the cell at various dilution rates. Maxon has explained the decrease in yield at high dilution rates as due to metabolism exceeding the rate that can be accommodated by aerobic mechanisms. Another point of interest in Maxon's work is that he observed cyclic changes in cell concentration, pH, and CO_2 production. This behavior has been explained as due to lag in response of the population to changes in pH or nutrient concentration.

Mukhopathyay and Ghose (76) conducted experiments with Saccharomyces cerevisiae in continuous culture, and concluded that "there is a specific steady state condition occurring at a particular dilution rate for a fixed fermenting volume. The steady state condition is fixed by the corresponding specific growth rate of the organism, the value of which may be determined, subject to the availability of kinetic data. Specific growth rate constant is not only a function of substrate concentration, but may also depend upon other simple physical variables such as volume, etc." The above statement appears to be completely different from other workers, and requires verification.

Tench and Morton (77) have compared enzyme kinetics with the activated sludge process, and proposed modifications considering the endogenous respiration of sludge. According to Tench and Morton, activated sludge and enzyme reactions differ in the sense that oxygen uptake occurs in the absence of substrate or in the presence of organic impurity in the sludge. Therefore, enzyme reaction has to be modified to account for this effect. Since autosynthesis and autooxidation are side reactions, they can be considered as proportional. If μ_o is the specific oxygen uptake rate, E is endogenous respiration rate, and μ_{oi} is the specific oxygen uptake rate due to the presence of impurities, then the Michaelis-Menten equation for oxygen uptake can be written as

$$(\mu_o - E) = \frac{(\mu_{om} - E)(S + i)}{k_o + (S + i)} \tag{68}$$

where k_o is the concentration of substrate at which μ_{om} is equal to 2 μ_o, and i is the concentration of impurities that will serve as additional substrate. When there is no substrate added, the oxygen uptake will be due only to the presence of impurities and to endogenous respiration.

$$(\mu_{oi} - E) = \frac{(\mu_{om} - E)\, i}{k_o + i} \tag{69}$$

By subtracting Equation 69 from 68

$$(\mu_o - \mu_{oi}) = \frac{k_o S \quad (\mu_{om} - E)}{(k_o + S + i)(k_o + i)} \tag{70}$$

Rearranging and substituting, from the above equations it can be shown that:

$$\frac{S}{(\mu_o - \mu_{oi})} = \frac{S}{(\mu_{om} - \mu_{oi})} + \frac{(k_o + i)}{(\mu_{om} - \mu_{oi})} \tag{71}$$

By plotting a graph of

$$\frac{S}{(\mu_o - \mu_{oi})} \text{ versus } S$$

a straight line relationship is obtained, the slope of which is equal to $\frac{1}{(\mu_{om} - \mu_{oi})}$ and the intercept is equal to $\frac{(k_o + i)}{(\mu_{om} - \mu_{oi})}$. Tench and Morton concluded that Equation 71 was applicable in their study conducted with activated sludge in a Warburg respirometer. The maximum oxidation rate was proportional to the number of bacteria actively metabolizing the substrate.

McKinney (78) has presented mathematical expressions describing the continuous flow process. He considered endogenous respiration in his derivation and also differentiated between active cell mass and total solids concentration. The growth of a microbial population in continuous culture has been assumed to be a function of limiting nutrient concentration but independent of cell concentration.

$$\frac{dx}{dt} = k_2 S \tag{72}$$

It can be seen that the above equation is independent of cell concentration, and is different from that proposed by others.

Pipes and Koutsoyannis (79) have conducted growth studies with Chlorella in which light is the factor limiting growth. When the concentration of Chlorella increases, mutual shading of the cells causes the light intensity to decrease and becomes a factor limiting growth.

Wilson (80) conducted experiments to study the growth of microbial populations. The growth kinetics proposed by Monod and Garrett were verified with the experimental data. The experiments were conducted using a Warburg respirometer and horizontally-revolving tube filters. It was found that the experimental data agreed more closely with Monod's (20) theory than with that of Garrett and Sawyer (18).

Fujimoto (81) developed theoretical equations relating substrate consumption and rate of growth which are different from those presented earlier. These equations were verified with experimental data obtained with baker's yeast, alcohol yeast, and Escherichia coli. The basic equations are given below:

$$-\frac{dS}{dt} = -\frac{\partial S}{\partial x}\frac{dx}{dt} - \frac{\partial S}{\partial P}\frac{dP}{dt} = s_r S \tag{73}$$

$$\frac{dx}{dt} = -Y\frac{dS}{dt} = \mu x \tag{74}$$

where P is the product formed other than microorganisms; s_r and μ are specific substrate consumption rate and specific growth rate, respectively. According to Fujimoto, both s_r and μ are functions of substrate concentration and cell concentration.

$$s_r = s_{rm}\frac{(x/S)}{\left[\frac{1}{k_s} + \frac{x}{S}\right]} \tag{75}$$

$$\mu = \mu_m \frac{(S/x)}{(k_s + S/x)} \tag{76}$$

According to Fujimoto (81) "Most research workers are of the opinion that growth is independent of cell concentration and consider that microorganisms can grow only at the expense of substrate. These workers have used the expression of Monod to relate growth and substrate consumption. While the equations, already presented, are suitable for special cases, Equations 73 and 74 give predictions of the entire course of bacterial growth and therefore yield better results."

McCabe (82) has used the kinetic theory proposed by Garrett and Sawyer (18) to describe the biological oxidation process. During the earlier phase of the process when there is excess substrate present, the growth is considered to be independent of substrate concentration. During this phase the growth rate is at its maximum value and can be described by the following expression:

$$\frac{dx}{dt} = \mu_m x \tag{77}$$

In the second phase substrate becomes a limiting factor for growth. The rate of growth is a function of substrate concentration and so also the rate of substrate utilization. It can be expressed mathematically as follows:

$$-\frac{dS}{dt} = k_1 S \tag{78}$$

There is a point of discontinuity in the growth curve separating the two phases. This theory was used by Eckenfelder (84) in developing activated sludge process design equations, but he modified Equation 78 to include solids concentration.

$$-\frac{dS}{dt} = k_1 x_a S \tag{79}$$

where x_a refers to the average solids concentration over the time interval considered. He also extended Equation 79 for a mixture of substrates, and presented a graphical method of evaluating the various parameters (84).

Weston and Stack (85) have presented a different approach for the prediction of the performance of

completely-mixed continuous biological systems from batch data. It is based on the equilibrium phase (stationary phase) of the BOD removal curve in batch systems. According to Weston and Stack, at the stationary phase the rate of BOD removal is equal to the rate of addition of substrate by death and lysis of cells. The equilibrium value of BOD has been found to be a function of initial BOD in the aeration mixture. Also, they have assumed that the BOD transfer rates occur in batch and continuous systems at the same resultant rate. The apparent coefficient of transfer rate is obtained from batch system studies, and used to predict the performance of the continuous system.

$$r_1 = \frac{(L_i - L_e)}{t_e L_e} \tag{80}$$

where L_i is the initial BOD, L_e is the BOD at equilibrium, t_e is the time required to reach equilibrium, and r_1 is the apparent coefficient of mass transfer. In using the transfer coefficient it is assumed that the continuous system operates in steady state at the equilibrium position attained in the batch system. The validity of this method will be discussed later in this dissertation.

Busch, et al. (86) have compared the performance of batch and continuous systems and concluded that so far as BOD removal is concerned, there is no marked difference in the two systems. But the clarifying ability of the two systems was different. Settleability in batch systems

appeared to be dependent upon sludge age, whereas in continuous systems the settleability was independent of sludge age but dependent upon applied surface loading.

Keshavan, et al. (87) concluded that the enzyme-substrate reaction and its equilibrium cannot be applied directly to biological reactions since equilibrium conditions will be maintained only in in vitro reactions, whereas in the case of a living cell the energy derived from biological oxidation is used to maintain the system in a state remote from equilibrium. From this point of view they have considered the reaction between bacteria and substrate as unidirectional resulting in the growth of new cells. Therefore, they have considered the overall reaction as follows:

$$\text{bacteria} + \text{substrate} \longrightarrow \text{increased number of bacteria} + \text{oxidized products}$$

The rate of forward reaction is proportional to the concentration of bacteria as well as substrate.

$$\frac{dx}{dt} = \mu \times S \tag{81}$$

It can be seen that Equation 81 is similar to that proposed by Garrett and Sawyer (18) and used by Eckenfelder (84). By replacing the substrate concentration in terms of yield (Y) and limiting concentration of solids (x_L) that can be supported by the substrate concentration, they have developed a growth equation which follows second order kinetics.

$$\frac{dx}{dt} = \frac{\mu}{Y} (x_L - x)\ x$$

$$= \mu' (x_L - x)\ x \qquad (82)$$

Keshavan, et al. have used the above equations of growth to predict the performance of a continuous flow process. Their results indicate that bacterial growth can be expressed by Equation 82, and they have used this growth equation for material balance equations of completely-mixed continuous flow systems. Further, they have shown that the steady state concentrations of substrate and cells obtained theoretically agree with their experimental data.

Martin and Washington (88) have considered substrate consumption for basal metabolism and the substrate introduced to the medium by death and lysis of cells. The material balance equation for substrate becomes

$$\begin{bmatrix}\text{rate of change of substrate}\\ \text{concentration in the reactor}\end{bmatrix} = \begin{bmatrix}\text{rate of increase}\\ \text{due to inflow}\end{bmatrix} -$$

$$\begin{bmatrix}\text{rate of decrease}\\ \text{due to outflow}\end{bmatrix} - \begin{bmatrix}\text{rate of decrease due to}\\ \text{consumption for growth}\end{bmatrix} -$$

$$\begin{bmatrix}\text{rate of decrease due to consumption}\\ \text{for basal metabolism}\end{bmatrix} + \begin{bmatrix}\text{rate of increase due}\\ \text{to death and lysis}\end{bmatrix}$$

$$\frac{dS}{dt} = DS_i - DS - \frac{\mu x}{Y} - B' x_A + B_1 B_2 B_3 x_A \qquad (83)$$

where B' is the specific rate of substrate consumption for basal metabolism, B_1 is the fraction of dead cells that lyse, B_2 is the amount of substrate released per unit

weight of lysed cells, and B_3 is the rate of death of cells per unit weight of active cells x_A. In a similar way the material balance equation for cells can be written as

$$\frac{dx}{dt} = \mu x - Dx - B_3 x_A \tag{84}$$

The material balance equations presented above were further developed to study the carbon transfer rate using radioactive C^{14}. Hetling, et al. (15) have used Equations 83 and 84 to show that consideration of the death rate of cells and substrate consumption for basal metabolism plays an important role in the determination of yield coefficient. In the absence of these two factors, Bx_A and $B_3 x_A$, the apparent yield is measured by the equation

$$Y = \frac{x_A}{(S_i - S)}$$

which is the same as Equation 22; whereas the true yield coefficient (Y_t) can be shown to be related to apparent yield coefficient (Y) by the following equation:

$$Y = \frac{1}{\frac{B_3}{DY_t} + \frac{1}{Y_t} + \frac{B'}{D}} \tag{85}$$

By substituting the mean residence time ($\bar{t}$) for 1/D, Equation 85 becomes

$$Y = \frac{1}{\frac{B_3 \bar{t}}{Y_t} + \frac{1}{Y_t} + B'\bar{t}} \tag{86}$$

or

$$\frac{1}{Y} = \frac{1}{Y_t} + \bar{t}\left[\frac{B_3}{Y_t} + B'\right] \tag{87}$$

Hetling, et al. (15) conducted experiments with pure cultures of Escherichia coli, Pseudomonas fluorescens, Alcaligenes faecalis, and Bacillus cereus in continuous flow experiments and determined the values of apparent and true yield coefficients. They concluded that the true yield coefficient of an organism is proportional neither to the COD nor to the free energy of substrate. The true yield coefficient can vary for different organisms for the same substrate. Marr, et al. (89) have also considered the requirement of substrate for basal metabolism. They have referred to basal metabolism as the "maintenance requirement" which can be defined as the consumption of carbon and energy source for purposes other than growth. Therefore the basic relationship between substrate consumption and growth is related by the following equation:

$$\frac{dx}{dt} + B'x = - Y \frac{dS}{dt} \tag{88}$$

At low dilution rates conventional equations for population density in a continuous flow process can be written as

$$x = \frac{Y\ S}{1 + B'/D} = \frac{X_{max}}{1 + B'/D} \tag{89}$$

Using Equation 89, Marr, et al. determined the specific maintenance rate for E. coli. Schulze (90) has used the expression of Teissier to predict the specific growth rate of microorganisms. According to him, the growth rate is given by the following equation:

$$\mu = \mu_m \left[1 - e^{-S/K_s} \right] \qquad (67)$$

which has already been presented. Schulze determined the specific growth rate from the dilution rate at steady state. The value of μ_m is taken as equal to the dilution rate at which washout of cells occurs. The experiments were conducted with a pure culture of Escherichia coli. In further work Schulze (91) applied the kinetic equations to compare the results obtained in actual treatment plants. He concluded that the application of the Michaelis-Menten equation (Monod) or the discontinuous growth equation (Eckenfelder) produce erroneous results, whereas the Teissier equation gives results that are in agreement with the experimental data.

3. Applications of the Continuous Flow Process

Thus far the various process rates and relationships for establishing overall kinetic theory for a completely-mixed continuous flow process have been discussed. A review of the literature indicates that there are different applications of the process both from the standpoint of waste treatment and other biochemical processes. These will be presented in the following portion of this chapter. Malek (92) has pointed out the possibilities of forming intentionally such systems as are necessary for maintaining a certain state of physiological properties; for example, the propagation activity connected with a cer-

tain optimum amount and mutual ratio of RNA and DNA, a certain enzyme complement, kinetics of variable processes, etc.

Continuous culture offers a unique method of obtaining microbial populations of uniform characteristics which is an essential requirement for biochemical research. Myers and Clark (28) used this process to obtain algal cultures for studying photosynthetic behavior. Herbert (4) has also pointed out that a continuous culture operating under steady state provides a source of microbial material in which all the microorganisms possess identical physiological properties. Gaudy, et al. (93) have discussed the necessity for the constancy of culture properties when one variable is studied. They used a continuous flow system to obtain sludge of constant property for studies on "qualitative shock loading." Since the biochemical response of a culture will change according to the composition of cells and their physiological state, the desirability of using continuous flow systems is becoming more and more important. Herbert (94) has conducted studies on the biochemical composition of cells at different dilution rates. He has shown that the RNA content of Bacillus cereus can vary between 3 and 30 per cent, depending upon the environmental conditions. He has also stressed the need for specifying the environment under which the cultures are grown; without doing this comparative studies are virtually meaningless. Ierusalimsky has reported in

his studies with continuous culture of Azotobacter vinelandii that the cell size, dry weight, and RNA content were found to increase with an increase in dilution rate or growth rate. Protein and DNA content were found to be relatively independent of growth rate (95). Postgate and Hunter have reported from their studies with Aerobacter aerogenes that large numbers of nonviable cells are found at very low dilution rates (95). Continuous cultures are applicable not only for the production of microorganisms in the field of biochemical research, but also for the production of baker's yeast and other commercial products having uniform characteristics.

Other applications include the separate treatment of industrial wastes which would otherwise produce a shock load on the microorganisms in municipal plants and consequently a reduction in the efficiency of the system. Coe (30) has used completely-mixed continuous flow process for the treatment of refinery wastes. The organic contaminants that are usually found in a refinery effluent include hydrocarbons, phenols, carboxylic acids, nitrogen compounds, and sulphur compounds. Pharmaceutical, food processing, and paper mill wastes were also treated by using continuous flow processes with BOD reduction efficiencies of 98.4 to 98.7 per cent (32). Maxon (8) has reported an interesting application in which gaseous hydrocarbons were converted to fatty acids by the oxida-

tive action of organisms such as Bacillus parafincus, B. methanicus, and B. ethanicus. These processes were patented by Standard Oil Company and Texaco Development Company. McKinney, et al. (34) have used the continuous flow process for the treatment of highly alkaline wastes without preneutralization. A design load of 60 pounds of BOD/day/1000 cubic feet of aeration volume has been used successfully. Abson and Todhunter (96) used a continuous flow process for the treatment of wastes from coke ovens and gas works. The objectionable materials in the above wastes include (a) phenols (monohydric and polyhydric), (b) thiocyanates, thiosulfates and cyanides, and (c) ammonia. They have designed a three-stage continuous flow process using organisms belonging to the genera Vibrio, Psuedomonas, Actinomyces, and certain strains of thiobacilli. The process was carried out in a specific sequence, since the presence of certain compounds will inhibit the stabilization of others. This is considered to be one of the best examples wherein the continuous flow process will have outstanding success. Another application has been presented by Evans and Kite (97) wherein they considered the removal of phenol alone with a single-stage continuous flow unit. They obtained a phenol removal efficiency of 99 per cent where the influent phenol concentration was 3750 mg/l.

Malek (97) has demonstrated that it is possible to ferment sulphite wastes, without diluting them in a two

or three-stage continuous flow process. It was also possible to produce fodder protein by direct fermentation of wastes. They have also shown that wastes from citric acid production and from yeast factories can be treated more efficiently in the continuous flow process than in the batch type process. In the treatment of the above wastes, cultures of sulfate-reducing bacteria were used in combination with bacteria decomposing betaine and protein.

Komolrit (99) studied the response of activated sludge to qualitative shock loading in both batch and continuous systems. When a sorbitol-acclimated culture of young cells was shocked with glucose solution the sorbitol removal was stopped until the glucose was metabolized in a batch system. In a continuous flow process no such effect was observed. When glucose and sorbitol were fed simultaneously in a continuous system, both compounds were removed simultaneously. He also conducted experiments with quantitative shock loading and slug dosage of one compound when the organisms were metabolizing another compound. He concluded that the response of a system to quantitative shock loads depends upon the organic load on the bio-mass. Also, he has shown that the ability of a system to accept a gradual shock load increases with increasing detention time. It can be inferred from his results that the continuous flow processes are more advantageous than batch processes, particularly where shock loads are expected.

Kincannon (23) employed the continuous flow process to study the effects of high salt concentration on the performance of the activated sludge process. He concluded that the physiological condition of the cells, or the cell age, plays an important role in the response of the cells to shock loads of sodium chloride. From the continuous flow studies, Kincannon observed that the predominating species present in the system determine the ability of the system to accept shock loads of sodium chloride. Further, he concluded that when the sludge grown on high salt concentrations are placed in fresh water there is a release of cellular compounds, indicating lysis, and the acclimation of sludge to sodium chloride results in the selection of species rather than a biochemical acclimation of prevailing species.

Novick and Szilard (100) conducted experiments to determine the rate of amino acid synthesis in bacteria, using a continuous flow process. They used a tryptophan-requiring strain of E. coli, with tryptophan as the growth-limiting factor. They presented an expression for the rate of production of a compound during transient conditions. If the response of the microorganisms is instantaneous due to change in the flow rate through the reactor, then the rate of production of a compound can be expressed by the following equation:

$$\sigma = \bar{x}_{11} D_1 + \left[\bar{x}_{22} D_2 - \bar{x}_{11} D_1\right] \left[1 - e^{-t/D_2}\right] \qquad (90)$$

where $\bar{x}_{11}$ and $\bar{x}_{22}$ are the steady state concentrations of cells at dilution rates of D_1 and D_2 respectively. σ is the rate of production of any compound at time, t, which in this case refers to organisms. They concluded from the experiments that the response of the culture was instantaneous within experimental limitations.

4. Economic Advantages of the Continuous Flow Process

The primary reason for the continued interest in the study of continuous flow processes is their economic advantages. Some of the economic considerations that have already been referred to in the literature will be discussed in the following section. The general advantage of all continuous flow processes is the susceptibility of the process to automation, which allows possibilities for reducing the operational cost. Another general advantage involves the reduction in number or size of the plants required for the same output of a compound or product (34).

Elsworth, et al. (101) have conducted experiments to compare the cost benefits of batch and continuous flow systems for a process involving the conversion of sorbitol to sorbose by the organism Acetobacter suboxydans. The cost comparison showed that a profit of $6,550 per annum could be achieved by using the continuous flow process. This does not mean that all continuous flow processes are profitable; a separate study of each process can decide the benefits of a particular process.

Sikyta and Slezak (102) have described the continuous cultivation of E. coli possessing high penicillin acylase activity. From their experiments they concluded that the output of cells in continuous cultivation is almost seven times higher than in the batch process.

5. Other Uses of Continuous Flow Process

Maxon (75) indicated that a multi-stage continuous flow process will be applicable where a heterogeneous substrate is gradually utilized at different rates for different components. Continuous flow processes have been used for adaptation processes. The adaptation of Torula utilis to sugars takes place in a relatively short time (8 days) as compared with the usual method of transfer and inoculation (22 days). Macura (103) used the continuous culture technique for studying soil microbiology. The action of microflora on organic compounds in soil can be considered as a continuous process in which nutrients are continuously absorbed by plants and microflora. During the growth phase of the plant and during the greater part of the vegitative period the medium is being enriched by organic and inorganic compounds eliminated from the roots.

Ordal and Palmer (104) used the continuous flow process for "enrichment technique." The steady state enrichment cultures differ from traditional enrichment cultures in that the continuous culture method is employed, and hence it is possible to maintain a relatively constant

environment in which one or more factors may be modified at will. Holme (105) has compared the batch type process with single and multi-stage continuous flow processes, and concluded that for yield measurements the continuous cultures offer distinct advantages over batch processes. When Aerobacter aerogenes was used for the production of 2,3-butanediol, it was found that a two-stage process with the first stage used to produce cells and the second used for the final product, gave the best yield.

Leal (106) used the continuous culture process to study the predominance of bacterial species that have different maximum growth rates. He used two organisms, Serratia marcescens and an unidentified organism isolated from activated sludge, which had different values for μ_m. He observed that the organisms were washed out of the culture vessel at a dilution rate greater than the value of μ_m determined in batch studies. He explained this result as due to the generation time being greater than μ_m by a factor of 1.443.

Jannasch (107) has described the use of continuous culture processes for the determination of starter populations for batch systems. The minimum population density which is regarded as starter population in batch culture is dependent upon growth rate and redox potential. The need for determining an optimum starter population has been explained as being due to an inhibitory

factor of the medium which is abolished at high population densities by metabolic activity of the organism. Humphrey and Reilly (108) have studied the fermentation of gluconic acid using Pseudomonas fluorescens in a continuous culture apparatus. The process involves the conversion of glucose to gluconolactone, and gluconolactone to gluconic acid. Since the conversion of gluconolactone to gluconic acid is a nonenzymatic process, no correlation was obtained between specific acid production rate and dilution rate or growth rate. Also, they used a two-stage continuous flow process, since glucose represses the formation of ketogluconic acid, whereas lactone does not. Therefore the first stage could be used to convert the glucose to the lactone form, leaving sufficient sugar to suppress the formation of ketogluconic acid. In the second stage a high pH can be maintained for rapid lactone hydrolysis, and the trace of sugar can be used to prevent ketogluconic acid formation. Holme and Zacharias (109) described the continuous production of gibberellic acid by a culture of Gibberella fujikuroi. They have also proposed a two-stage process, one for the mycelial growth, and the second for the production of gibberellic acid.

6. Total Recirculation of Sludge in the Continuous Flow Process

Since one experiment conducted in this research is concerned with total cell recirculation, it is appropri-

ate to review some of the work that has been accomplished in this aspect. In total recirculation, no sludge is wasted except that which leaves in the effluent without settling, and that removed for sampling. This resembles "total oxidation systems" or "extended aeration systems" which have been studied previously by several workers. The total oxidation process consists of comminution of sewage, long-term aeration, final settling, and return of the settled sludge to the aeration tank. Primary settling and sludge digestion are eliminated, while the aeration tank is enlarged to provide the required aeration period. For a conventional total oxidation system, the aeration period is usually twenty-four hours with a BOD loading of 30 pounds/day/1000 cu. ft. Tapleshay (110) has reported on the operation of one such system. He concluded that the volatile content of the sludge in a total oxidation system reaches a value of 50 per cent or even lower. This compares with about 85 per cent volatile content of the sludge in a conventional activated sludge process.

Kountz and Forney (111) have reported an accumulation of 0.122 pounds of sludge per day in the aeration tank. The wasting of 0.122 pounds of sludge every day resulted in operation of the system at equilibrium. They also found that the percentage of nitrogen in the cellular material did not change with time. Busch and Myrick (36) studied both batch and continuous flow processes without sludge

wasting. They concluded that it is theoretically impossible to maintain indefinitely a growing culture with specific physiological characteristics. Evolution in the form of selection and mutation will bring about changes. In their experiments it was not possible to obtain a food-population equilibrium in either batch or continuous flow system even after 103 days of operation. Washington and Symons (112) have also reported that extracellular polysaccharides build up in sludges, and that they are resistant to biological degradation. They concluded that total oxidation systems cannot be operated without sludge buildup. Washington and Symons (112) also conducted experiments on volatile sludge accumulation in total oxidation systems, and concluded that solids accumulate at a rate of 10 to 15 per cent of the ultimate BOD removed. The rate of accumulation might be different for different systems. They concluded that organic nitrogen cannot be taken as a measure of active bacterial population, since the inert solids were found to contain carbohydrates, organic nitrogen, and fatty acids. Ludzack (113) has conducted experiments on a bench scale total oxidation system. The following conclusions have been reported from his studies: the volatile content of the sludge dropped from 75 to 55 per cent, and mixed liquor suspended solids showed a parallel decrease. A highly nitrified effluent was obtained at normal temperatures. The settleability

of the sludge was affected due to the flotation of denitrified sludge. Respiration rates of the sludge were found to be low, indicating a small proportion of active sludge. Finally, he concluded that a weekly wastage of a certain fraction of suspended solids might result in the successful operation of the system with reduction in effluent solids concentration.

In the preceding paragraphs there have been presented some of the major developments and applications of the continuous flow process, which is the subject of the research reported herein. It is believed that the historical development presented above will provide the basis for further developments that are to be presented in the following chapters.

CHAPTER IV

MATERIALS AND METHODS

1. Experimental Design

(a) Studies without Recirculation

Continuous flow units were run at various dilution rates with glucose as growth-limiting substrate. Glucose was selected as the substrate for several reasons; first, a considerable amount of work has been published by other workers using glucose as substrate; secondly, soluble substrates are believed to present the most difficult problems in industrial waste treatment. Also, soluble substrates will not interfere with the determination of biological solids, which is an important kinetic parameter. The dilution rates studied cover a wide range, from 1/24 hr^{-1} to the dilution rate at which a complete washout of cells occurs. The corresponding mean residence times were 24, 18, 12, 6, 4, 3, 2, $1\frac{1}{2}$, 1, and $\frac{1}{2}$ hours, respectively. The units were run at each dilution rate for a time sufficiently long so that a reliable statistical estimation of the steady state parameters could be made.

The parameters that were used in this study include suspended solids concentration, chemical oxygen demand,

and carbohydrate concentration in the effluent. Samples were collected from both the mixed liquor and the effluent from the reactor, so that any deviations from complete mixing conditions could be detected and corrected. Microscopic examinations were conducted at each dilution rate to study the effect of dilution rate on population dynamics. The concentration of dissolved oxygen was also measured at each dilution rate. The specific respiration rate of washed cells was determined to estimate the active population at each dilution rate. At the end of each dilution rate experiment, washed cells from the mixed liquor and the filtered effluent were collected and stored in a freezer (-20° C.) for further analyses, such as sludge composition and composition of the effluent.

The growth parameters μ_m, Y, and k_s were determined at each dilution rate by shake-flask experiments. Duplicate runs were made in determining the above parameters to check the consistency of the results obtained. Oxygen uptake determinations were also made concurrently at certain dilution rates to study the correlation between growth rate and oxygen uptake rate. Warburg respirometers were used for determining the oxygen uptake rate.

Two substrate concentrations were used in the studies without recirculation; they were 1000 mg/l and 3000 mg/l. The procedures were identical in both cases, as it was intended to study the effect of inflow substrate con-

centration on overall process kinetics. When glucose was used at 3000 mg/l, the inorganic salts and buffer concentrations were proportionally increased to maintain the same relative concentrations as that of the 1000 mg/l glucose feed.

(b) Studies Employing Recirculation of Sludge

Only the 1000 mg/l concentration of glucose was used in this study. Dilution rates of 1/6, 1/4, 1/3, and 1/2 hr^{-1} were employed with volumetric recirculation ratio of 0.25. The dilution rates refer to overall flow through the reactor including the return sludge. The effluent from the reactor was allowed to settle, and the settled concentrated sludge was recirculated to the reactor. The concentration factor used was 1.50; this factor is the ratio of the concentration of sludge in the recylce to that in the mixed liquor. The same parameters were measured as in the case of experiments wtihout recirculation. The concentration of biological solids in the return sludge was measured at frequent intervals.

(c) Studies Employing Total Recirculation of Sludge

In these studies all the settled sludge was recycled to the aeration vessel. The separation of sludge was accomplished by introducing a settling tank in the system and withdrawing the sludge from the bottom of the settling tank. The flow of return sludge was kept the same

as that of the inflow medium. The overall dilution rate studied was 1/4 hr^{-1}. This corresponds to a detention time of four hours. The substrate concentration in the inflow medium was maintained at 1000 mg/l glucose. In addition to measuring the parameters mentioned previously, volatile suspended solids were also measured in this experiment.

The units at the beginning of each experiment were seeded with primary effluent collected from the sewage treatment plant at Stillwater, Oklahoma. This waste water is primarily domestic in nature. The sludge was allowed to grow in the reactor until sufficient solids concentration (approximately 500 to 1000 mg/l) had built up. Then the continuous flow of medium was begun. Samples were collected for analyses, beginning one day after the start of the continuous addition of nutrients.

2. Description of the Equipment

(a) Studies without Recirculation

Figure 8 shows the general arrangement of the equipment used for the continuous flow studies. The reactor (B) used in these studies was made of glass, and had a capacity of 2.50 liters. Constant volume is maintained by means of an overflow weir; the effluent from the reactor flows into the settling tank (C) from which it is wasted. The temperature of the reactor was maintained at $25^{o} \pm 2^{o}C$

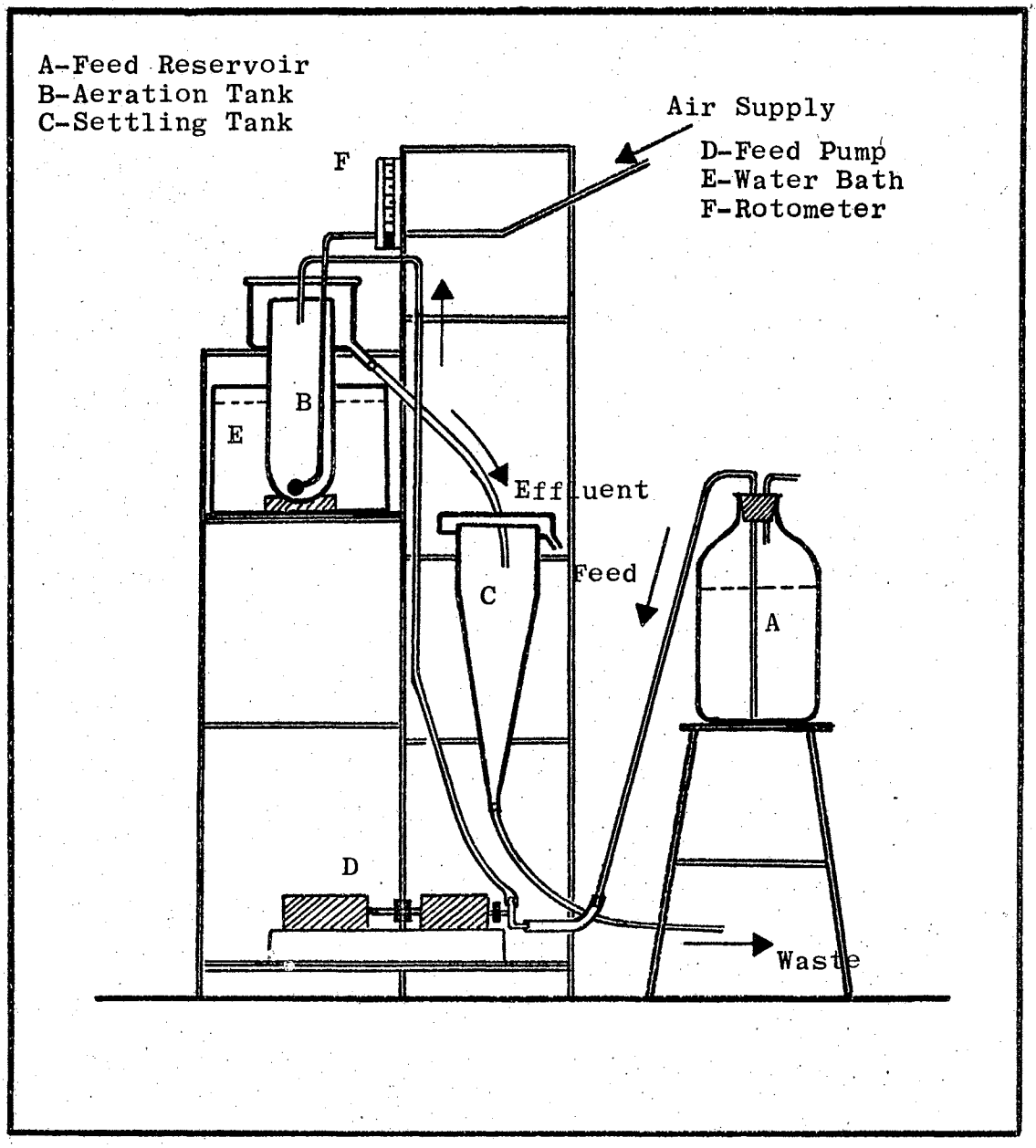

Figure 8. Schematic Representation of the Continuous-Flow Completely-Mixed Reactor.

by means of a water bath (E). Feed solution was pumped to the reactor by means of reciprocating pumps (D) manufactured by Milton Roy Company ("Minipump" Model MM 4-C-48R). Feed solution was prepared in a pre-sterilized 20-liter glass carboy (A) and connected to the feed line. It was found necessary to change the feed reservoir at least every 36 hours in order to prevent contamination in the reservoir. Since it was not possible to sterilize the feed solution due to the precipitation of the inorganic salts during sterilization, the above procedure was adopted during the entire study. The concentration of the substrate in the feed was checked frequently to detect any errors in making up the feed solution.

During the early part of the study it was found that the feed line was becoming contaminated. In order to prevent this, a standby pump was operated at all times, and the feed lines were sterilized by pumping a dilute solution (1 per cent by volume) of "Clorox." The feed lines were then freed from Clorox solution by pumping water through them several times before they were reused. Another difficulty in the operation of continuous flow processes is the growth of organisms along the side and bottom of the reactor. To prevent any such growth, the sides of the reactor were scrubbed by means of a rubber scraper at least twice a day.

Agitation and mixing were accomplished by diffusing air through the mixed liquor. Growth was also observed

on the diffuser stones or glass diffusers, and frequent change of diffusers was found necessary. The air supply was maintained at 1600 cc/min/liter with a pressure of 30 psi.

The accomplishment of completely-mixed conditions depends not only upon mixing, but also on the design of the reactor vessel. Since a homogeneous suspension of biological solids is maintained in the aerator, it is necessary that the outlet ports are so designed that no settling of solids occurs in the outlet device. Figure 9A shows the type of reactor used during the early part of this research. It is apparent from the figure that settling of solids in the outlet tube can disturb the completely-mixed conditions. It was found that the effluent solids concentration was always lower than that inside the reactor. A modification of the design of the reactor was proposed, and Figure 9B shows the second model used. Even though the performance of this reactor was better than the previous design, it was decided that a larger opening in the wall of the reactor or a weir type overflow device would be ideal for this study. The final design of the reactor is shown in Figure 9C. The mixing conditions in this reactor were verified by using a solution of methyl red at pH 2.0 at different flow rates. The concentration of dye solution was measured by optical density at a wave length of 570 mμ. Figure 10

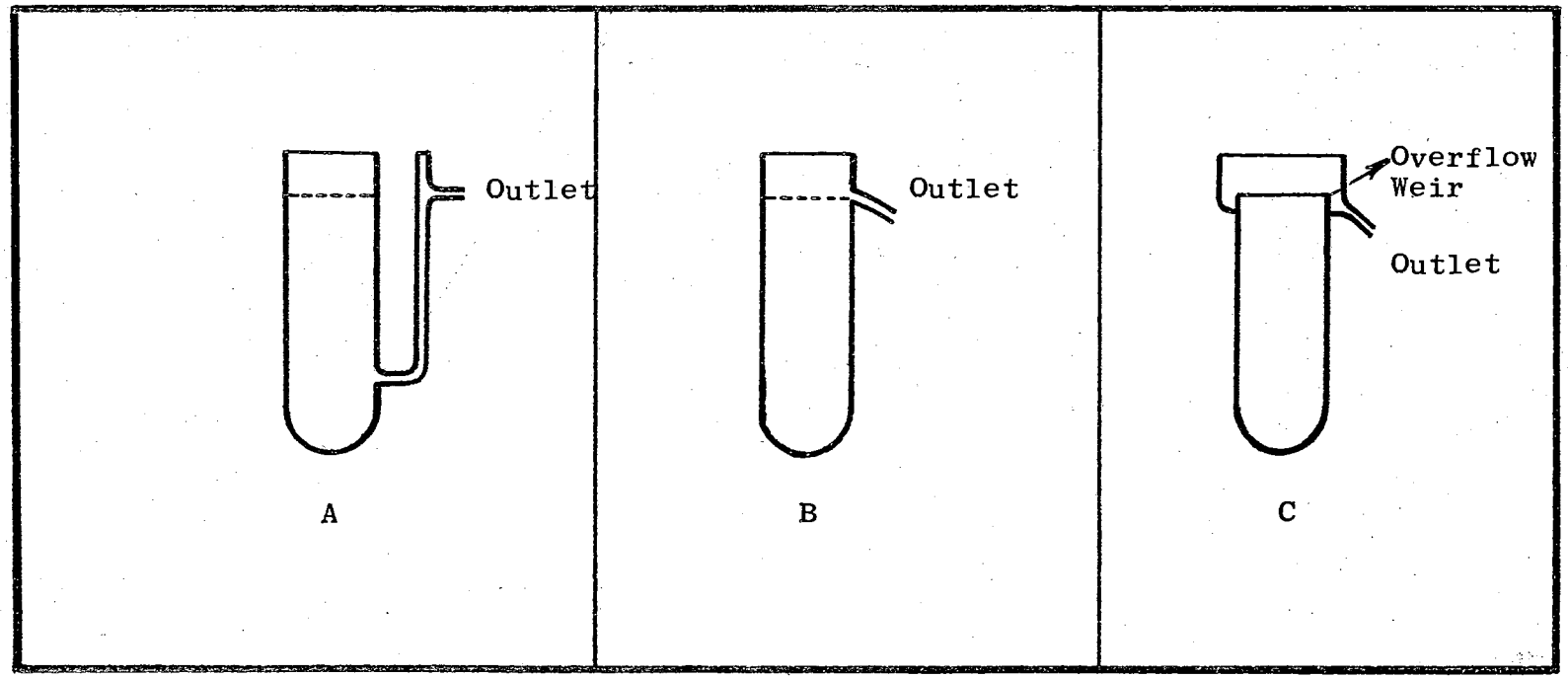

Figure 9. Figure Showing the Developments in Reactor Design.

shows the absorption spectrum of the methyl red solution at pH 2.0. Figure 11 shows the theoretical and observed dilution curves at different dilution rates. It can be seen from the graphs that mixing conditions in the reactor were very close to the ideal, and the theoretical equations for a completely-mixed system can be applied to the reactor shown in Figure 9C.

(b) Studies Employing Recirculation of Sludge

The operational procedure and arrangement of the equipment are similar to those for the experiments without recirculation, the difference being only in the recycling portion of the equipment (Figure 12). The settled sludge from the settling tank was drawn into a secondary aeration vessel. The secondary aerator was intended to maintain a homogeneous supply of the return sludge and to prevent anaerobic conditions. The return sludge is pumped to the reactor by means of a Sigma Motor Pump ("Zeromax," Inc.). The concentration of biological solids in the return sludge tank was maintained constant by collecting a definite volume (5 liters) in the settling tank and withdrawing a particular volume sufficient to maintain the concentration at 1.5 times the concentration of biological solids in the reactor. Figure 12 shows the recycle portion of the experimental equipment. The dilution rates studied were 1/6, 1/4, 1/3, and 1/2 per hour. It was not possible to conduct studies at a higher dilution rate than 1/2 per

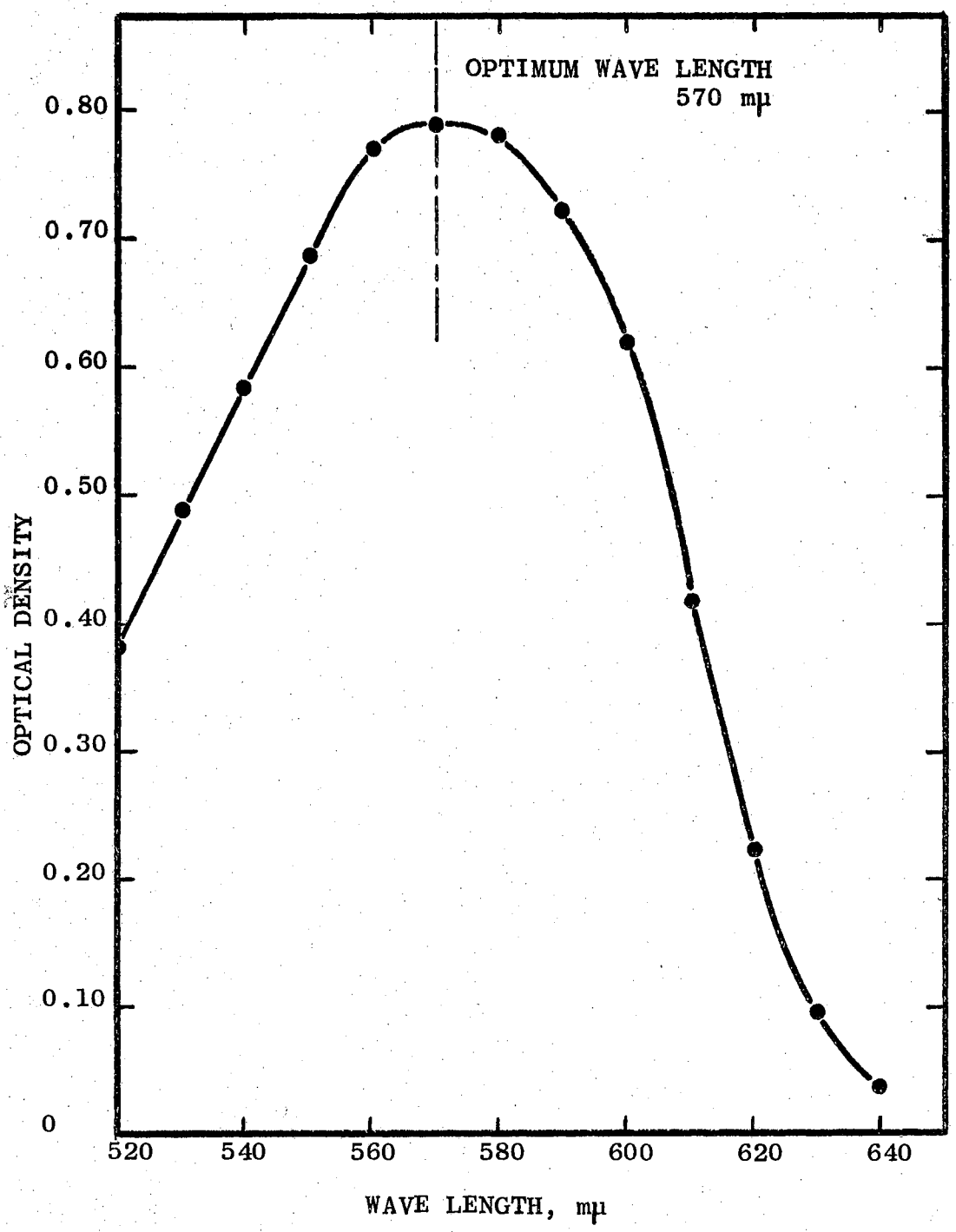

Figure 10. Absorption Spectrum of the Methyl-Red Solution at pH = 2.0 (Coleman, Model-D Spectrophotometer).

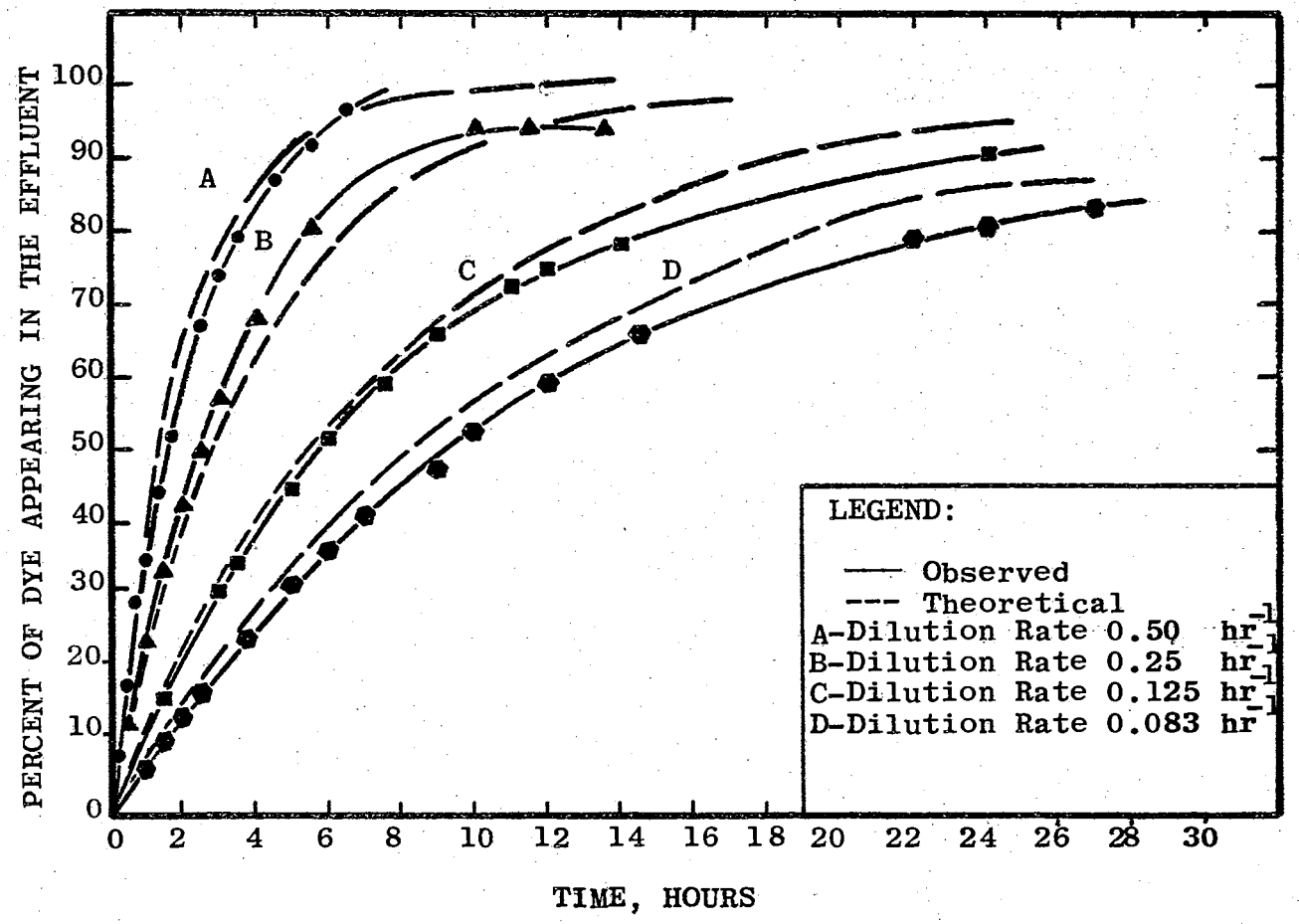

Figure 11. Theoretical and Observed Dilution Curves for Methyl-Red Dye Solution at pH 2.0.

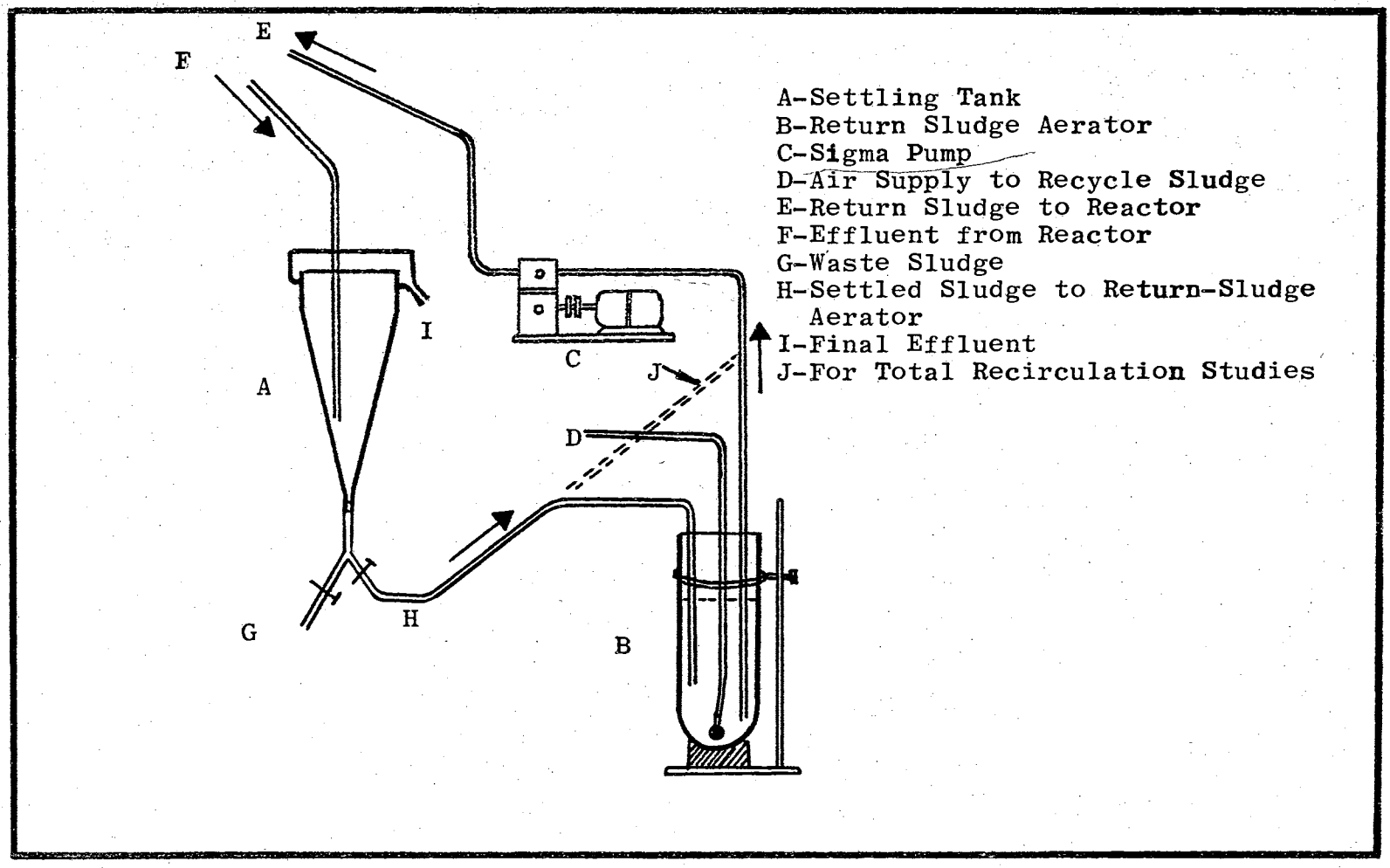

Figure 12. Schematic Representation of the Recycle Portion of the Completely-Mixed Continuous-Flow Activated Sludge System.

hour, since proper settling and separation of sludge did not occur to maintain the biological solids concentration at 1.50 times that in the reactor.

(c) Study Employing Total Recirculation

This study was designed to investigate the performance of a total recirculation system under continuous flow conditions. The apparatus used was the same as for the studies with recirculation, except that the return sludge aeration tank was omitted from the circuit. Instead, the settling tank was connected directly to the reactor through the Sigma pump. The flow path is shown by the broken line in Figure 12. It was not necessary to include a return sludge aeration tank, since there was not enough accumulation of sludge in the settling tank to create septic conditions. The overall dilution rate studied was 1/4 per hour, and due to this high dilution rate the settled sludge was rapidly recycled to the reactor.

3. Analytical Procedures

(a) Steady State Parameters

Determinations of biological solids were accomplished using the Millipore filter technique. Adequate samples of mixed liquor and collected effluent were filtered through previously dried and tared Millipore filters having a pore size of 0.45 μ. The filters were then oven-dried (103^{o}C.), cooled in a dessicator, and weighed. From the differences

in weight the concentrations of biological solids were determined. The mean value of the solids concentrations in the mixed liquor and in the effluent was taken as the steady state solids concentration. Frequently it was found necessary to centrifuge the samples before filtering, so that filtration could proceed at a rapid rate. The solids were transferred from the centrifuge tube to the filter with great care in order to minimize the loss of solids during transfer. For the determination of volatile suspended solids the biological solids were transferred to crucibles and placed in a muffle furnace (600^{o}C.) for 30 minutes. The crucibles were cooled in a dessicator, and weighed. The percent ash content and percent volatile content of the sludge were determined.

The filtrates from the samples were collected and used for further analyses; these included the determination of COD and the concentration of carbohydrate. Chemical oxygen demand determinations were carried out according to Standard Methods (114). Carbohydrate content was measured by the anthrone method, as described by Gaudy (115). The anthrone test measures the total carbohydrate content, and is not specific for glucose. The color developed by anthrone with standard glucose solutions and with the unknown samples was measured in a Coleman Model D spectrophotometer at a wave length of 540 mμ. The carbohydrate contents were measured and reported as glucose in this study. Standard glucose samples were run with every car-

bohydrate determination to minimize the errors due to variations in experimental conditions.

In addition to the determinations of biological solids concentration, chemical demand, and carbohydrate, pH and temperature measurements were also taken at every sampling time. It was observed that the pH changed slightly with dilution rate. Since it was intended to maintain constant pH for all dilution rates, the buffer concentrations were increased at high dilution rates in order to maintain the pH of the culture at approximately 7.0. For each dilution rate the concentration of dissolved oxygen in the mixed liquor was also measured. A Jarrell-Ash dissolved oxygen analyzer was used for this analysis.

(b) Growth Parameters

The growth parameters μ_m, k_s, and Y were determined by shake-flask experiments. Twelve 250 ml erlenmeyer flasks were marked appropriately for the different substrate concentrations to be used in the experiment. Substrate concentrations of 50, 100, 200, 300, 500, and 800 mg/l glucose were used. The complete medium included glucose, inorganic salts, and buffer solution in the same proportion as they were used in the feed solution for the chemostat. The composition of the medium for 1000 mg/l glucose as substrate is given in Table I.

TABLE I

COMPOSITION OF GROWTH MEDIUM FOR 1000 mg/l GLUCOSE AS GROWTH-LIMITING SUBSTRATE

Constituents	Concentration	
Glucose	1000	mg/l
Ammonium sulfate, $(NH_4)_2SO_4$	500	mg/l
Magnesium sulfate, $MgSO_4.7H_2O$	100	mg/l
Ferric chloride, $FeCl_3.6H_2O$	0.50	mg/l
Manganous sulfate, $MnSO_4.H_2O$	10.00	mg/l
Calcium chloride, $CaCl_2$	7.50	mg/l
KH_2PO_4	527.00	mg/l
K_2HPO_4	1070.00	mg/l
Tap water	100	ml/l

Tap water is added in order to provide trace elements. Six flasks with 500 mg/l glucose were used in addition to the six flasks of different concentrations mentioned above. Sixty ml of the medium were placed in each flask marked with appropriate concentrations of the growth-limiting factor. The flasks were seeded with 0.50 to 1.0 ml of the mixed liquor from the continuous flow reactor and placed on a reciprocating shaker. To study the relationship between oxygen uptake rate and growth rate, 40 ml of the medium and seed were put in Warburg flasks, one flask being used for each concentration of the growth-limiting factor mentioned above. The Warburg and shaker apparatus were started simultaneously, and both were operated at 90 ± 5 oscillations per minute.

The growth rate in each flask in the shaker was determined by measuring the optical density at a wave length of 540 mμ. Oxygen uptake measurements were also made at different times to determine oxygen uptake rate. The six additional flasks with the same concentration of growth-limiting factor (500 mg/l) were removed at different times during the exponential growth phase. The contents of the flasks were filtered through Millipore filters, and the filtrates were used for determining COD. From the concentration of COD the yield coefficient (Y) was determined. The yield coefficient is reported as the weight of biological solids produced per unit weight of chemical oxygen demand utilized. For determining the specific growth rate at each concentration of growth-limiting factor the optical density data were convered to biological solids concentration. Rao (116) has correlated the relationship between optical density and the concentration of biological solids for activated sludge grown on glucose. Figure 13 shows the relationship between optical density and the concentration of biological solids. This figure was used for the conversion of the optical density data to biological solids concentrations. The biological solids concentrations were plotted against time on semilogarithmic paper for each concentration of growth-limiting factor. The specific growth rates (μ) for each substrate concentration were determined from the straight line portion of the curve by the following relationship:

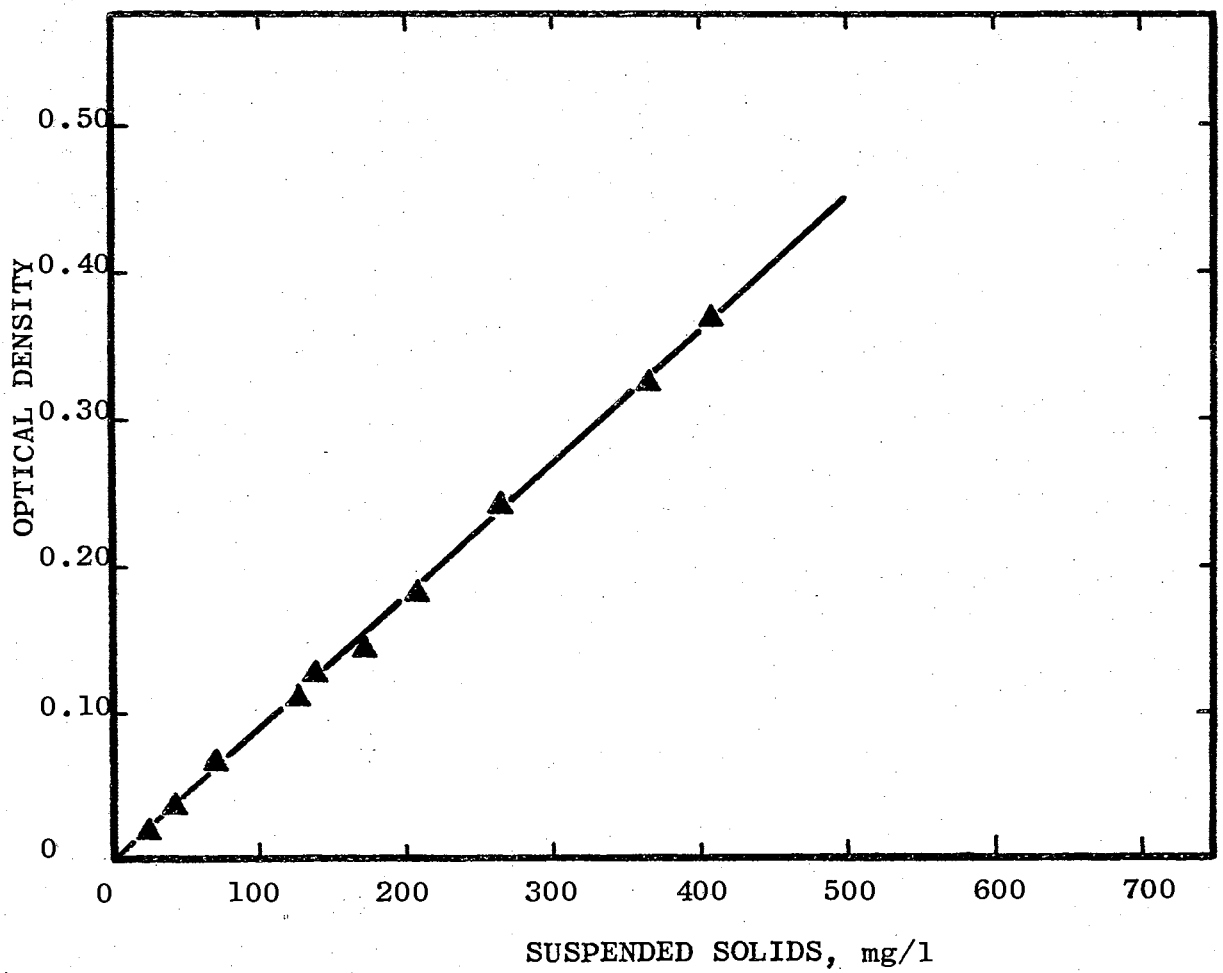

Figure 13. Relationship Between Optical Density and Concentrations of Suspended Solids. (after Rao) .

$$\mu = \frac{(\log x_2 - \log x_1)2.30}{(t_2 - t_1)} \qquad (91)$$

The values of μ_m and k_s were determined by plotting a curve of μ versus the concentration of growth-limiting factor. The value of μ at the point where the curve becomes asymptotic to the x-axis gives the value of μ_m. The value of k_s can also be calculated from the same graph by determining the substrate concentration at which the value of μ is 1/2 μ_m. In order to verify these values of μ_m and k_s, graphs similar to Lineweaver and Burk plots were also drawn for each run and the values of μ_m and k_s were determined. Similar determinations were also made with the cumulative oxygen uptake data and the growth parameters so determined were compared with those from growth rate data. The major difficulty in the determination of growth parameters was observed when the flocculation of cells interfered with the determination of biological solids concentration by optical density. When flocculation occurred the run was discontinued and repeated again. For the experiments on total recirculation it was never possible to eliminate flocculation. Therefore the growth rate was measured by concentration of suspended solids determined by the Millipore filter technique. Since large volumes of culture are required for this method, 2-liter erlenmeyer flasks were used for each substrate concentration. The experiments were started with 800 ml of culture in each flask, and aeration was accomplished using carborundum diffusers.

(c) Unit Activity

Unit activity is defined as the mg of oxygen consumed per gm of sludge per hour in the absence of exogenous substrate. Since only living cells consume oxygen, this may be taken as a measure of the fraction of active cells in the total mass. A sufficient amount of mixed liquor was collected from the reactor, and the sludge was washed twice with 1.0 M phosphate buffer solution, and then resuspended in buffer solution. The washing was done by separating the sludge using a high speed centrifuge and resuspending the sludge in the buffer solution. A "Waring blender" was used to resuspend the cells. The blender was run for a duration of one minute. This procedure was repeated for a second time before the cells were finally suspended. The suspended solids concentration in the washed cell suspension was determined by the Millipore filter technique. The oxygen uptake rate of 40 ml of the cell suspension was determined using a Warburg respirometer. Triplicate samples were run for each dilution rate, and the mean value was taken as the representative oxygen uptake. From the oxygen uptake data and the solids concentration the unit activity of the suspension was determined.

At the termination of each run the mixed culture in the reactor was centrifuged and filtered through Millipore filters. The filtrate was collected, labeled, and stored in the freezer for further analysis. The separated cells

were washed with buffer, as described previously, resuspended in buffer solution, and stored in the freezer. The above procedure was repeated for all experiments.

The stored cells were later analyzed for carbohydrate and protein content. The defrosted sample of cell suspension was homogenized in a Waring blender, and the suspended solids concentration was determined by using Millipore filters. For the analysis of protein and carbohydrate content of cells, a sonifier was used for homogenization, and analyses were made on aliquot samples. The carbohydrate content was determined by the anthrone method (115). The protein content was determined by the biuret method, as described by Gaudy (115). Protein and carbohydrate contents were reported as percent of cell mass. Protein concentrations were measured using albumin as standard. With every analysis, standard albumin solutions were also run to minimize experimental variations.

The data on biological solids concentration and chemical oxygen demand, collected during steady state operation, were analyzed statistically to estimate population means, standard deviation, and coefficient of variance. These estimated values were used for kinetic analysis of the completely-mixed continuous flow process.

CHAPTER V

RESULTS

1. Relationship between Substrate Concentration and Rate of Growth

Establishment of an appropriate relationship between the concentration of growth-limiting factor and specific growth rate becomes an essential prerequisite for the kinetic study of biological processes. Lack of such a unifying relationship has resulted in a number of mathematical expressions to describe a single process, namely, the growth of microorganisms.

(a) Studies without Recirculation

The relationship between the concentration of growth-limiting factor and specific growth rate (μ) for a mixed culture maintained at steady state is shown in Figure 14. The specific growth rate values at each substrate level were determined by shake-flask experiments. In the same figure are also shown the theoretical plots obtained by Equation 14. Figure 15 shows the same data plotted in a different way. Equation 14 can be rearranged as follows:

$$\frac{1}{\mu} = \left[\frac{k_s}{\mu_m}\right]\frac{1}{S} + \frac{1}{\mu_m} \qquad (92)$$

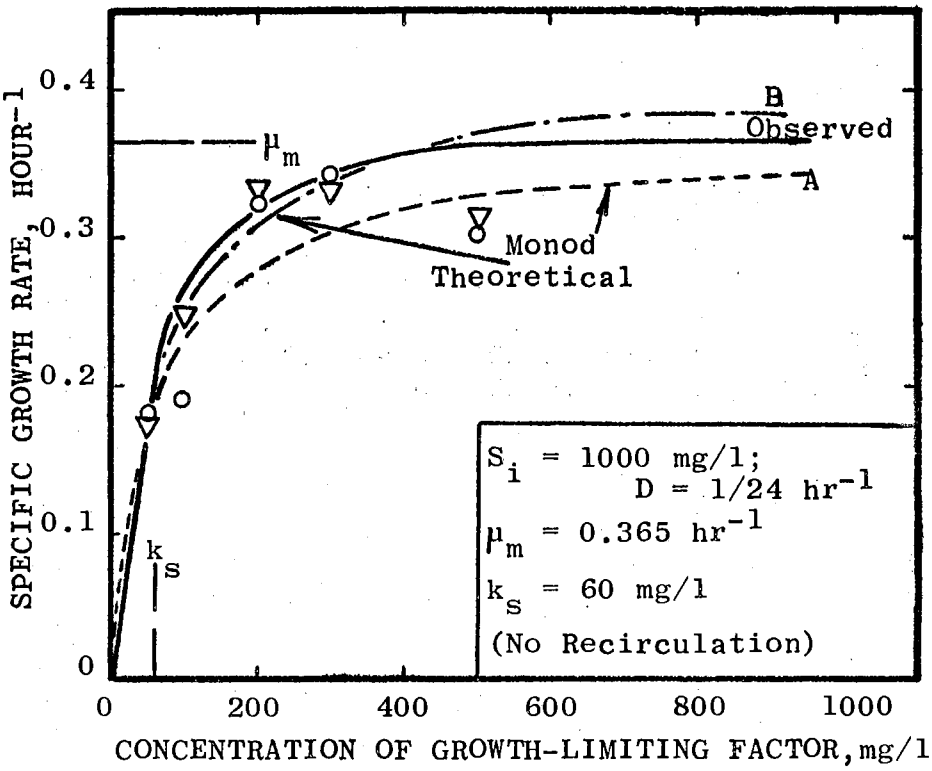

Figure 14. Relationship between μ and S.

A-Theoretical Curve when μ_m = 0.365; k_s = 60
B-Theoretical Curve when μ_m = 0.416; k_s = 68

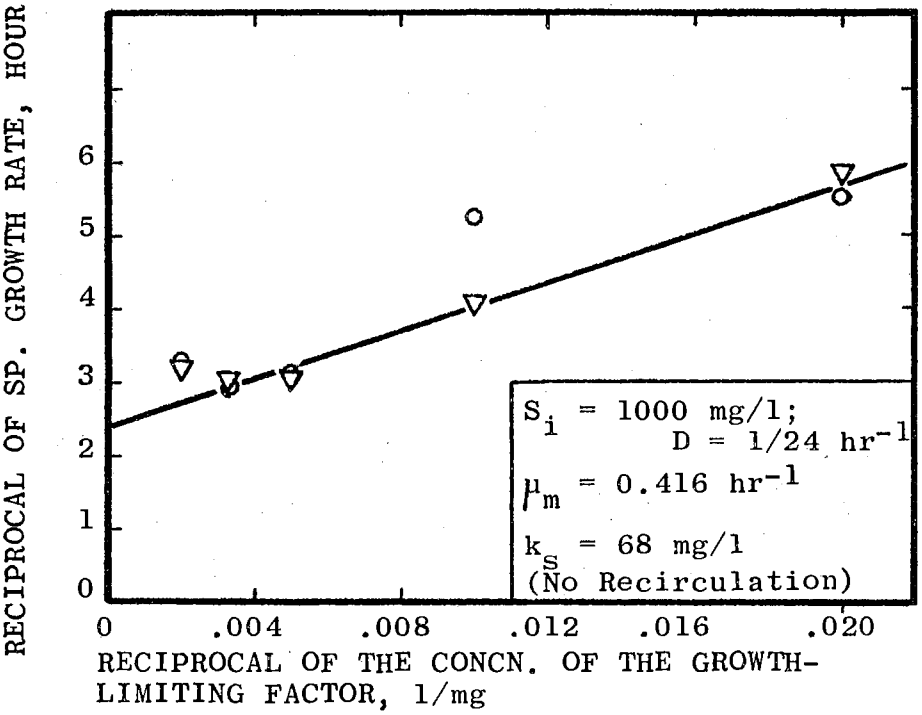

Figure 15. Relationship between $1/\mu$ and 1/S.

A plot of $1/\mu$ versus $1/S$ is shown in Figure 15. It can be seen from these figures that Equation 14 proposed by Monod (20) closely agrees with the observed values. The values of μ_m and k_s were obtained from Figures 14 and 15, and it can be seen that they do not agree closely. This may be attributed to the fact that the estimation of μ_m in Figure 14 is only approximate, since the curve is asymptotic to the abcissa. Similarly, the estimation of k_s in Figure 14 is also approximate, since it depends upon the value of μ_m. Two theoretical curves are shown in Figure 14. Curve A was plotted from the μ_m and k_s values obtained from Figure 14, and curve B was plotted from values taken from Figure 15. It is evident from the figure that the use of the growth parameters (μ_m and k_s) estimated from Figure 15 gives a better fit than do the values taken from Figure 14. This supports the previous statement that the estimation of μ_m and k_s from Figure 14 is only approximate. It can also be seen that neither of the theoretical curves agrees totally with the experimental curve. Figures 16 to 29 show results of similar experiments using cells harvested from a steady-state continuous flow activated sludge unit which was operated at different dilution rates. In all cases the inflow glucose concentration to the steady-state units was 1000 mg/l and recirculation of sludge was not employed. Examination of Figures 16 through 29 indicates that, in general, the theoretical plot of Equation 14 agrees with

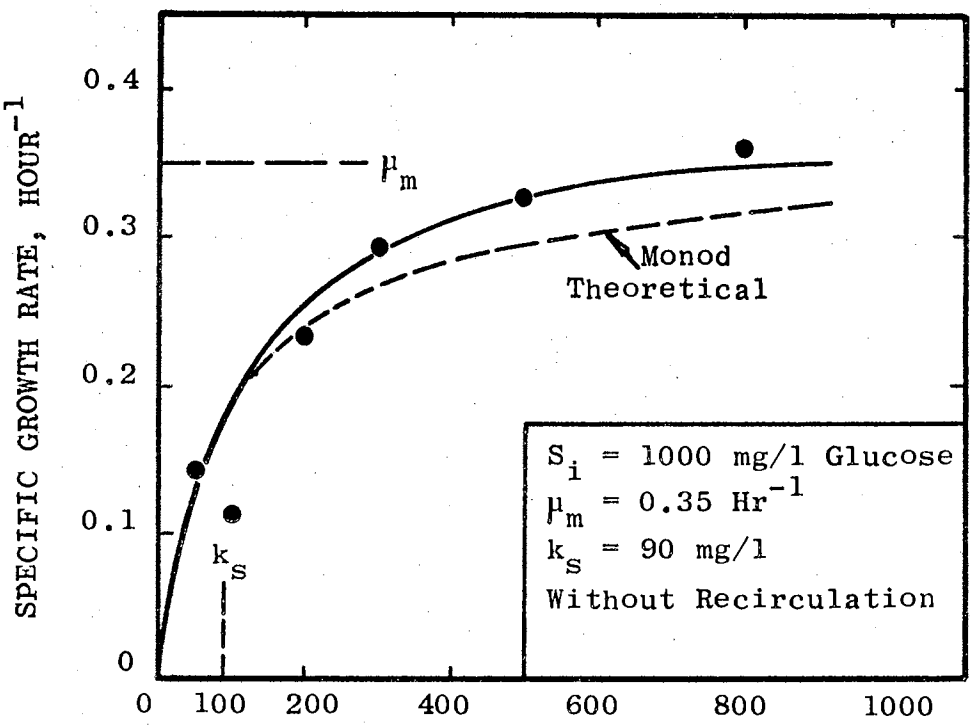

Figure 16. Relationship between μ and S at D = 1/18 Hour^{-1}.

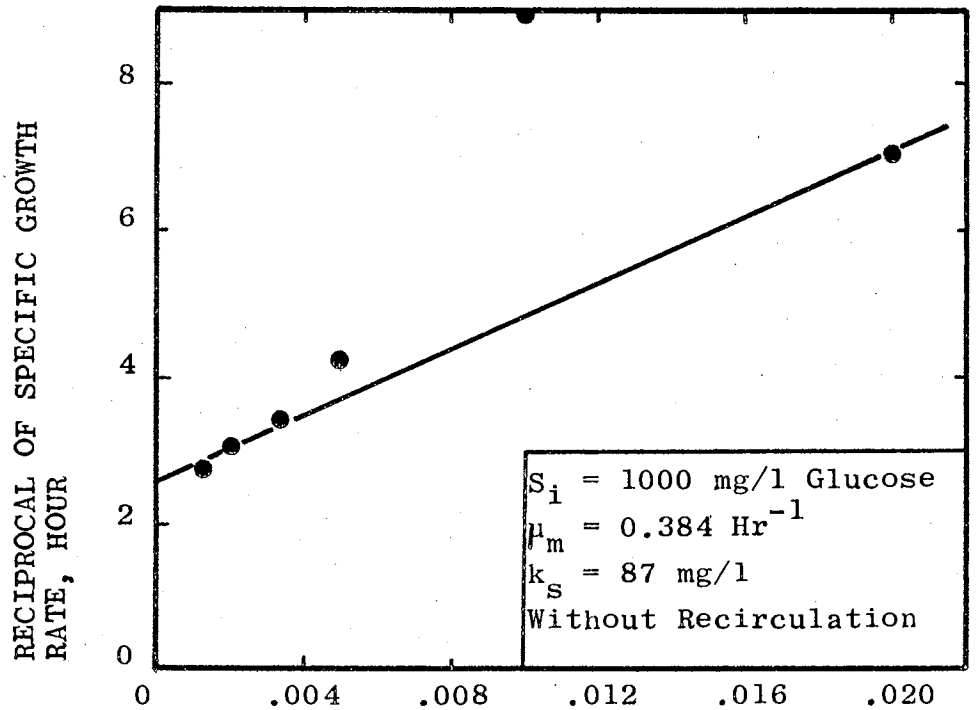

Figure 17. Relationship between $1/\mu$ and $1/S$ at D = 1/18 Hour^{-1}.

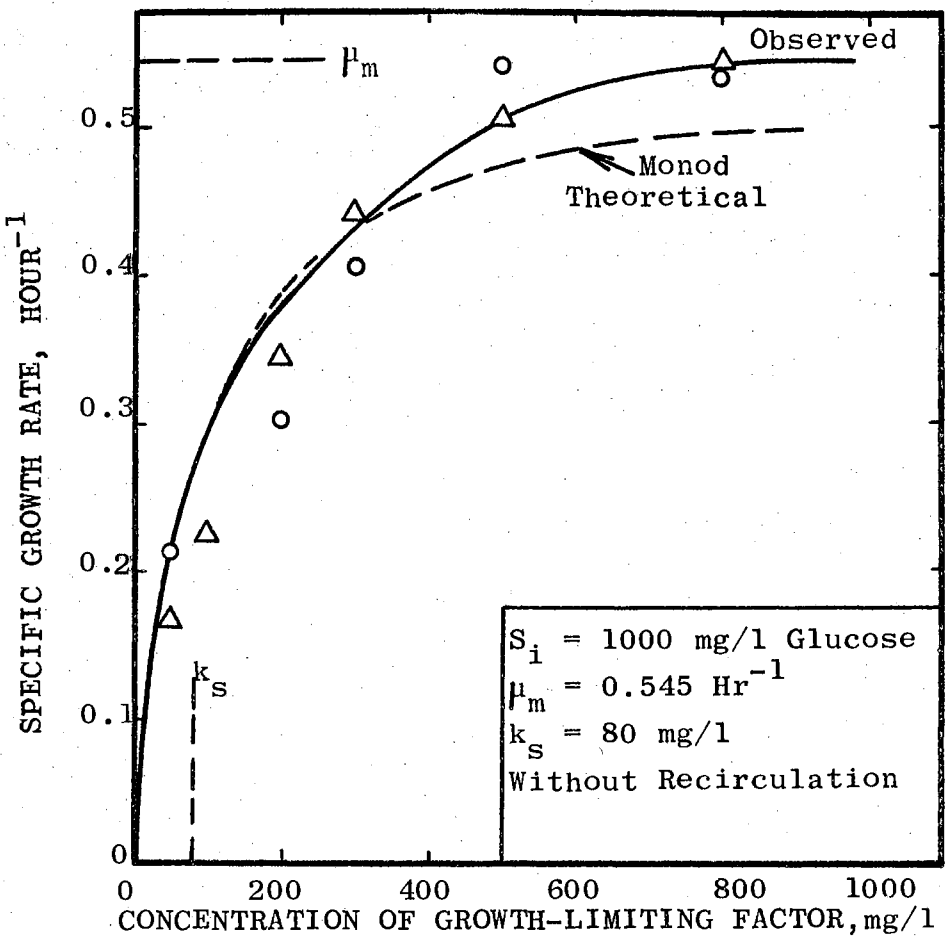

Figure 18. Relationship between μ and S at D = 1/12 $Hour^{-1}$.

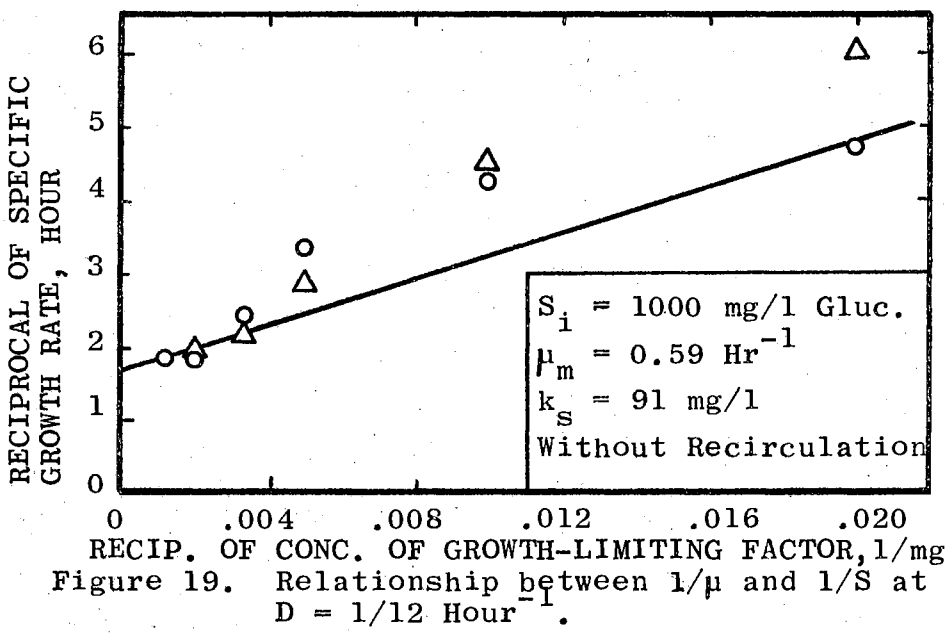

Figure 19. Relationship between $1/\mu$ and 1/S at D = 1/12 $Hour^{-1}$.

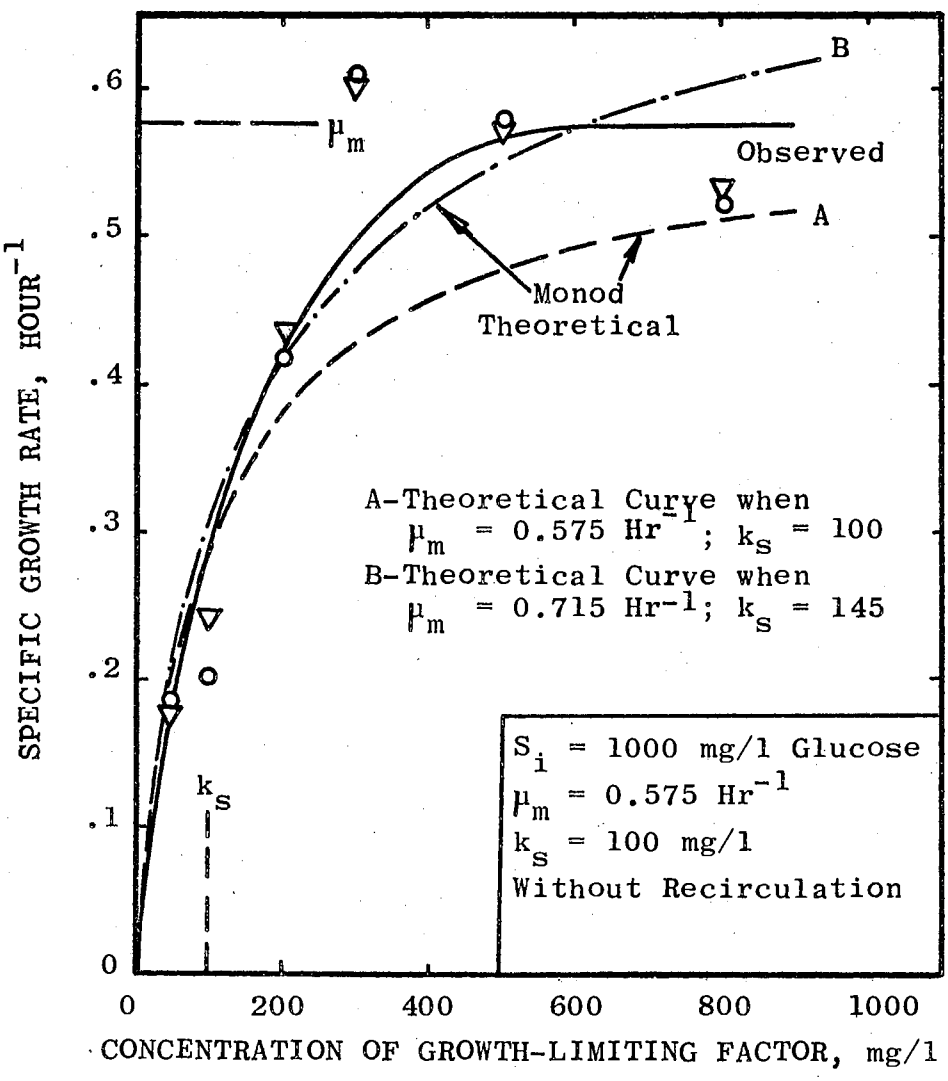

Figure 20. Relationship between μ and S at D = 1/6 Hour^{-1}.

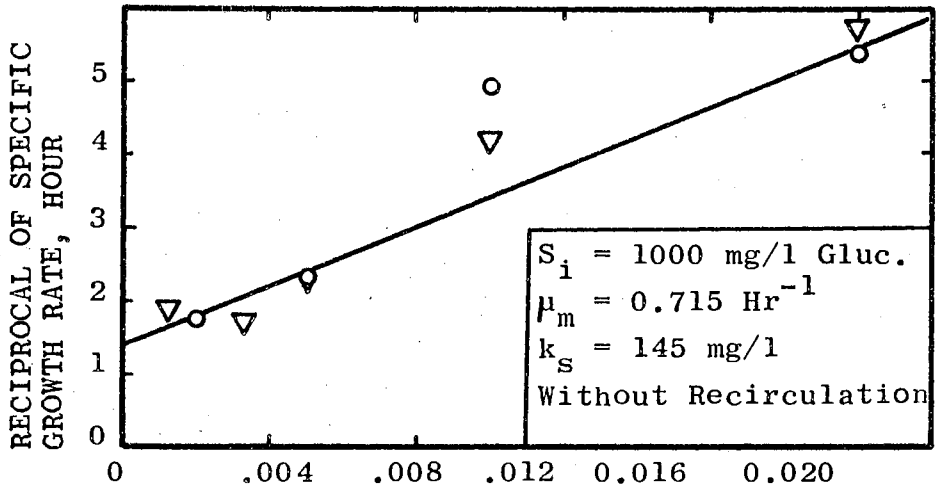

Figure 21. Relationship between $1/\mu$ and 1/S at D = 1/6 Hour^{-1}.

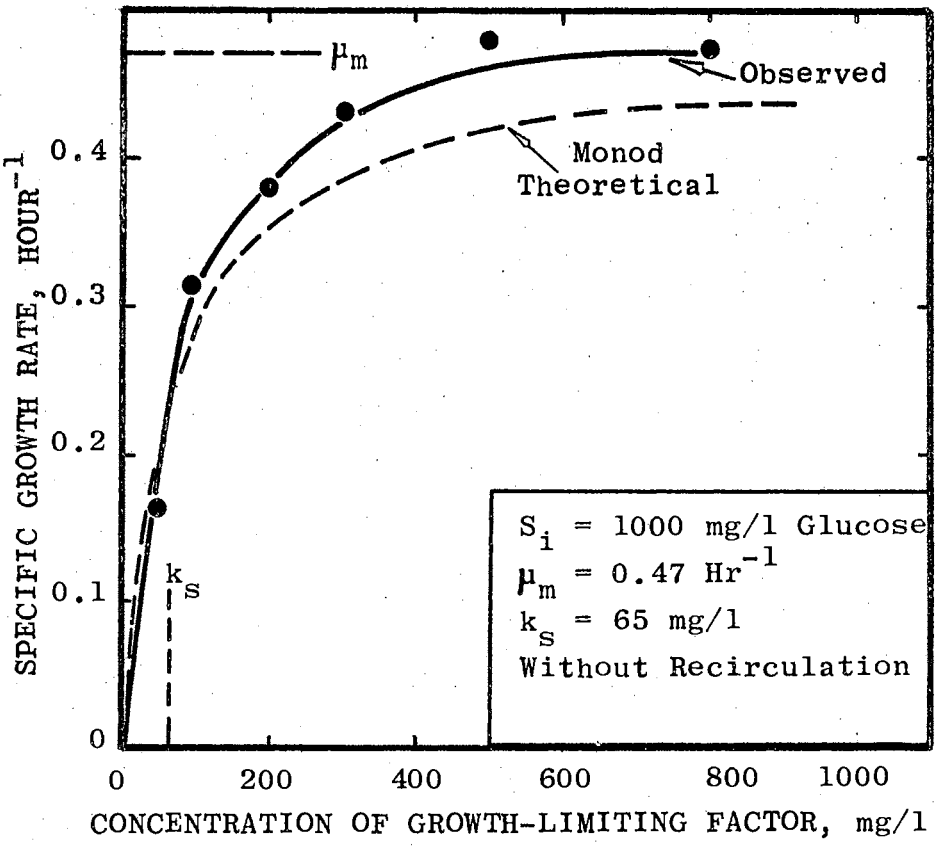

Figure 22. Relationship between μ and S at $D = 1/4\ Hour^{-1}$.

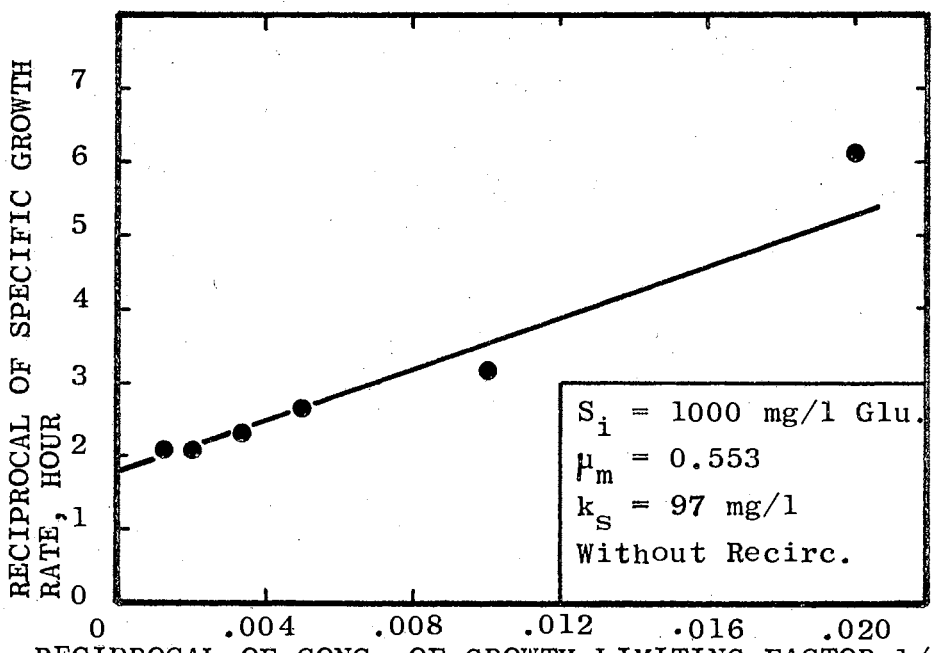

Figure 23. Relationship between $1/\mu$ and 1/S at $D = 1/4\ Hour^{-1}$.

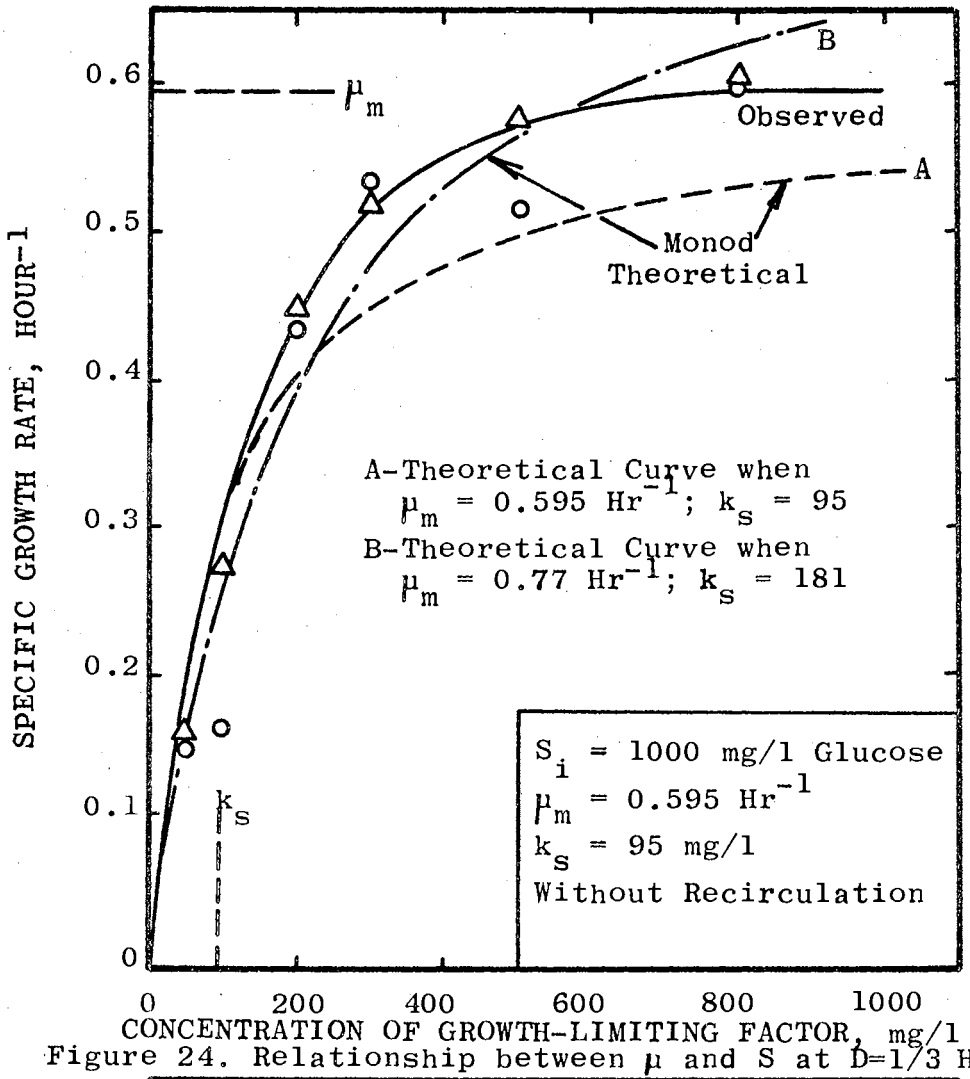

Figure 24. Relationship between μ and S at $D=1/3\ Hr^{-1}$

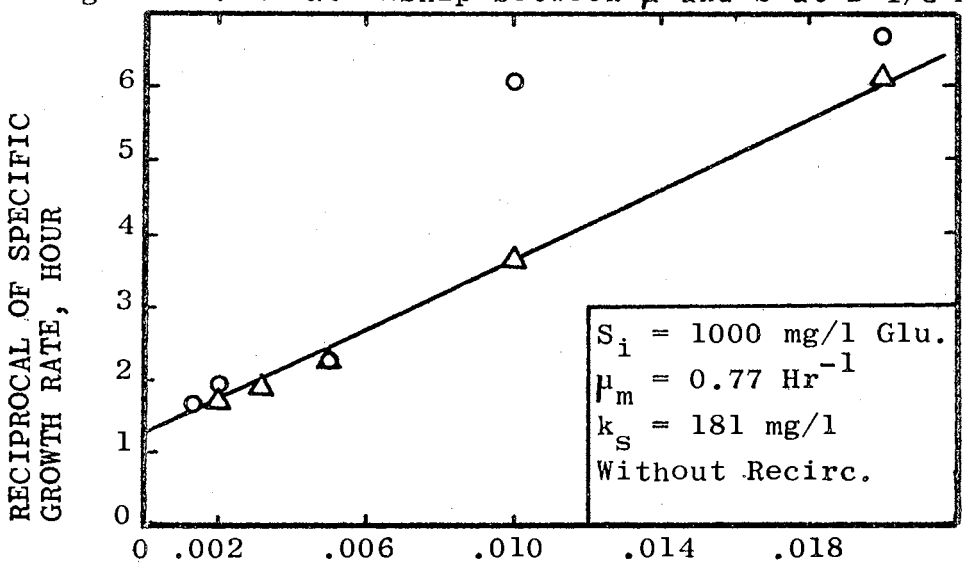

Figure 25. Relationship between $1/\mu$ and $1/S$ at $D = 1/3\ Hour^{-1}$.

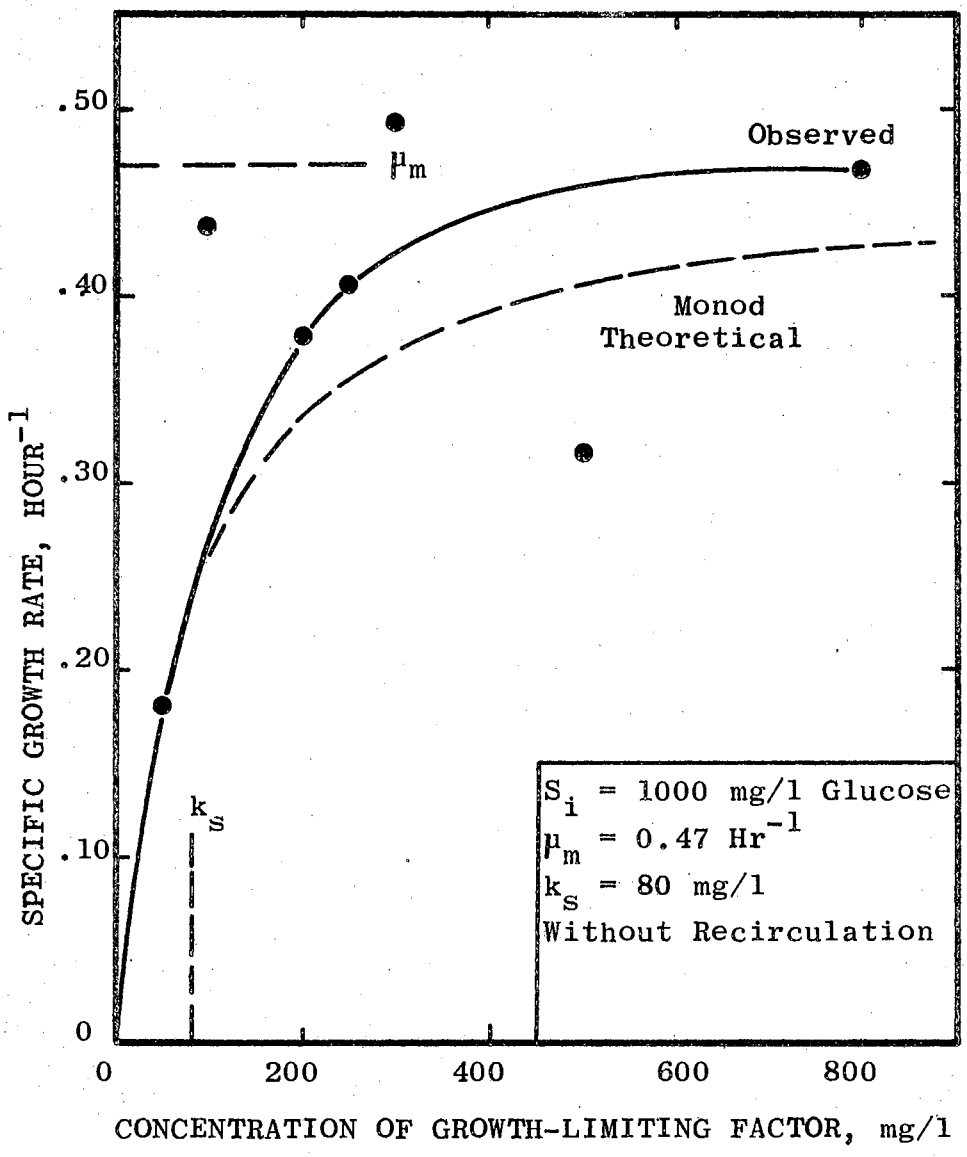

Figure 26. Relationship between μ and S at D = 1/2 $Hour^{-1}$.

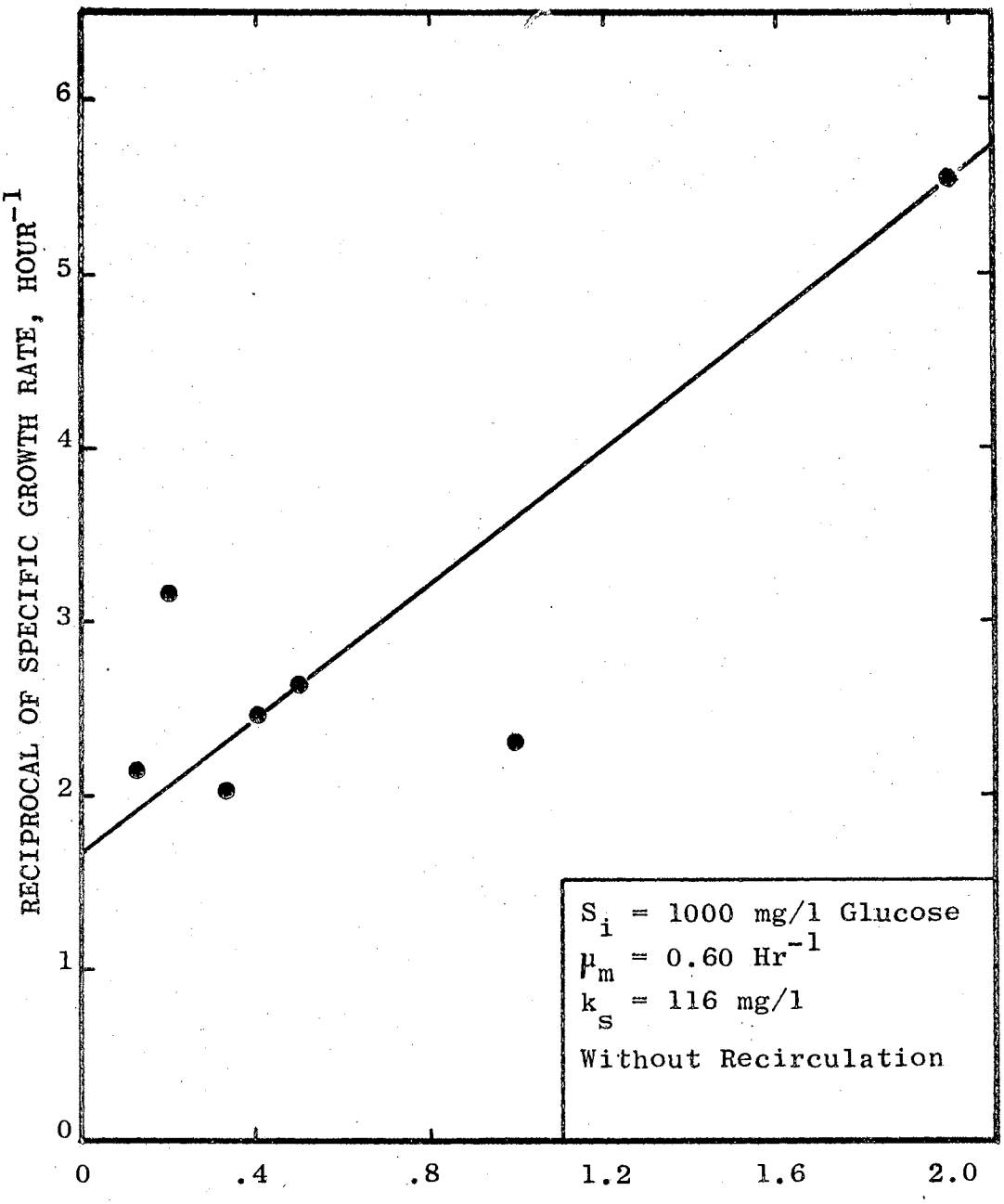

Figure 27. Relationship between $1/\mu$ and $1/S$ at $D = 1/2$ Hour^{-1}

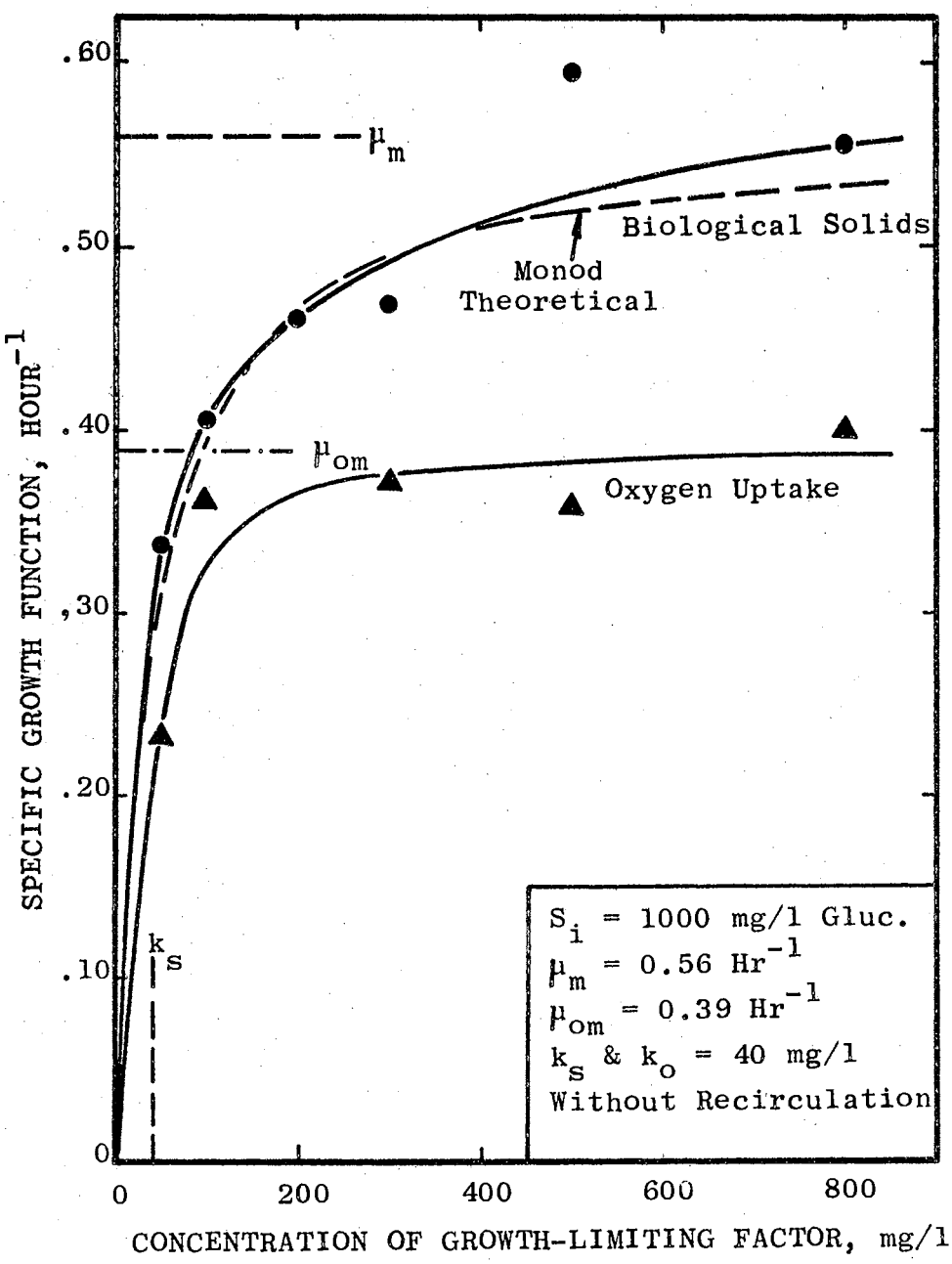

Figure 28. Relationship between μ, μ_o, and S at D = 1/1.50 $Hour^{-1}$.

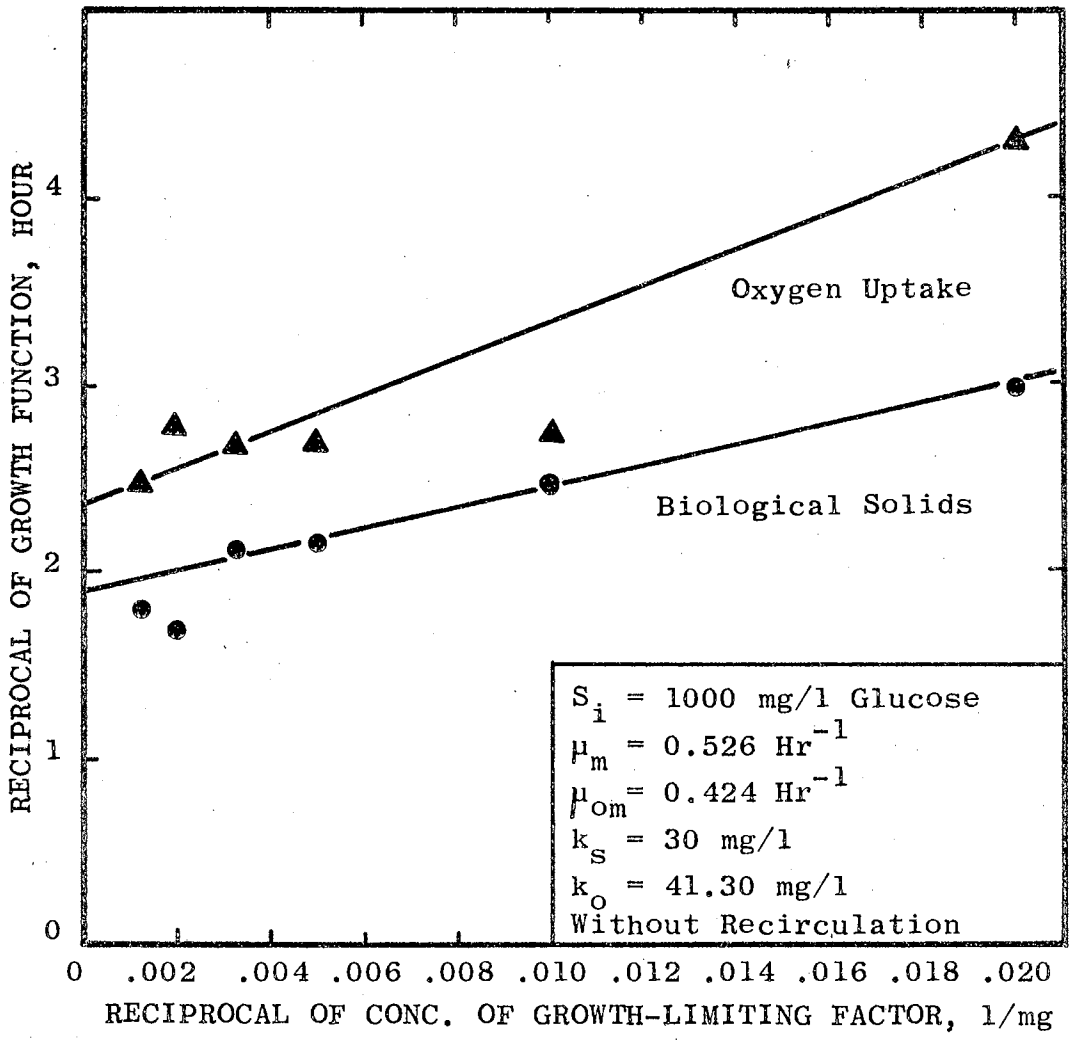

Figure 29. Relationship between $1/\mu$, $1/\mu_o$, and $1/S$ at D = 1/1.50 $Hour^{-1}$.

the observed values of μ; this is true particularly at lower concentrations of the carbon source. It can also be seen that the theoretical curve plotted using constants obtained from the Lineweaver and Burk plots yield a better correlation with the observed values than the constants estimated from the plot of μ vs. S.

A summary of the values of μ_m, k_s, and Y_B values is given in Table II. It can be seen from the table that the value of μ_m is not a constant, but varies with the dilution rate. It will be shown later that μ_m increases with dilution rate up to a certain flow rate, and then decreases. It can also be seen that the values of k_s vary independently of dilution rate. The relationship between Y_B and dilution rate will be discussed later in this chapter.

In order to test the suitability of the theoretical expressions developed by other research workers the theoretical curves were compared with the observed values. Figure 30 shows the plots of theoretical curves calculated from Equation 54 proposed by Moser (19) for different values of λ. It can be seen from the figure that the observed values (plotted as closed circles) of μ fit the curve for $\lambda = 1.0$ better than any of the other curves. It is also interesting to note that when λ equals 1.0, Equation 54 reduces to the one proposed by Monod. Figures 31 through 33 show similar plots for cells grown at other dilution rates. The specific growth rate values, for different values of λ, were calculated using μ_m and k_s values from

TABLE II

GROWTH PARAMETERS OBTAINED FROM BATCH EXPERIMENTS ACCORDING TO MONOD'S THEORY

(S_i = 1000 mg/l Glucose; No Recirculation)

Dilution Rate	Specific Growth Rate, Hr^{-1}						μ_{m1}	k_{s1}	μ_{m2}	k_{s2}	μ_m	k_s	Yield Coefficient (Y)	
	Concentration of Substrate, mg/l													
Hr^{-1}	50	100	200	300	500	800	Hr^{-1}		Hr^{-1}		Hr^{-1}		Batch	Cont.
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
1/24	0.177	0.220	0.327	0.338	0.309	-	0.365	60	0.416	68	0.390	64	0.46	0.552
1/18	0.142	0.112	0.234	0.293	0.326	0.36	0.350	90	0.384	87	0.367	89	0.42	0.692
1/12	0.189	0.274	0.324	0.424	0.524	0.538	0.545	80	0.588	91	0.567	86	0.37	0.675
1/6	0.181	0.222	0.428	0.605	0.573	0.529	0.575	100	0.715	145	0.645	123	0.46	0.636
1/4	0.162	0.316	0.380	0.432	0.480	0.475	0.470	65	0.555	97	0.513	81	0.46	0.685
1/3	0.156	0.221	0.444	0.528	0.549	0.603	0.595	95	0.77	181	0.683	138	0.48	0.615
1/2	0.180	0.436	0.378	0.492	0.315	0.466	0.470	80	0.60	116	0.540	98	0.48	0.558
1/1.5	0.336	0.405	0.460	0.470	0.595	0.555	0.560	40	0.53	30	0.545	35	0.48	0.785

Note: μ_{m1} and k_{s1} - obtained from the graph of μ vs. S

μ_{m2} and k_s - obtained from the graph of $1/\mu$ vs. $1/S$

μ_m and k_s - are the mean values of μ_{m1} and μ_{m2}, and k_{s1} and k_{s2}

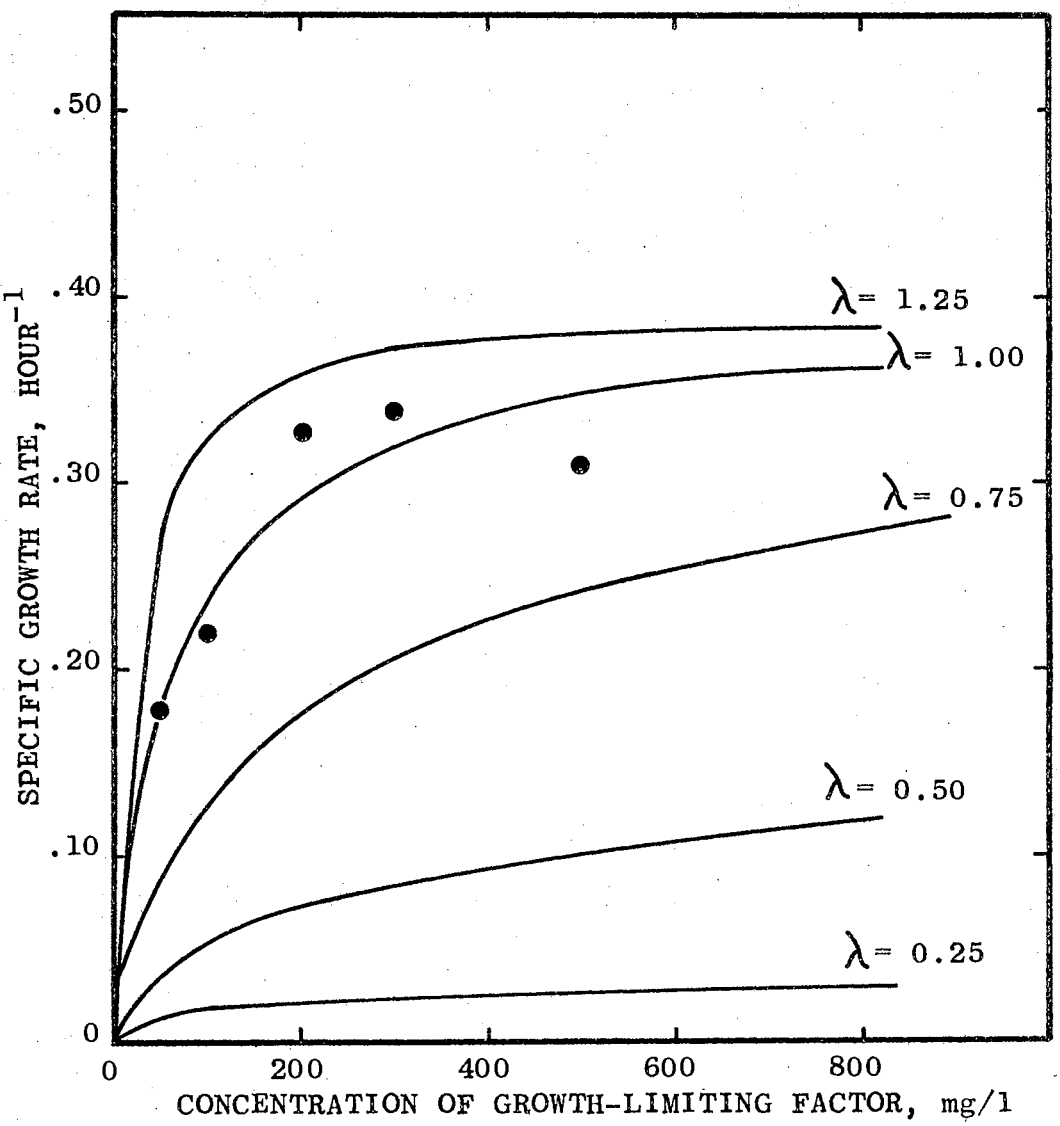

Figure 30. Relationship between μ and S according to the theory of Moser at D = 1/24 Hr^{-1}: (S_i = 1000 mg/l Glucose; without Recirculation)

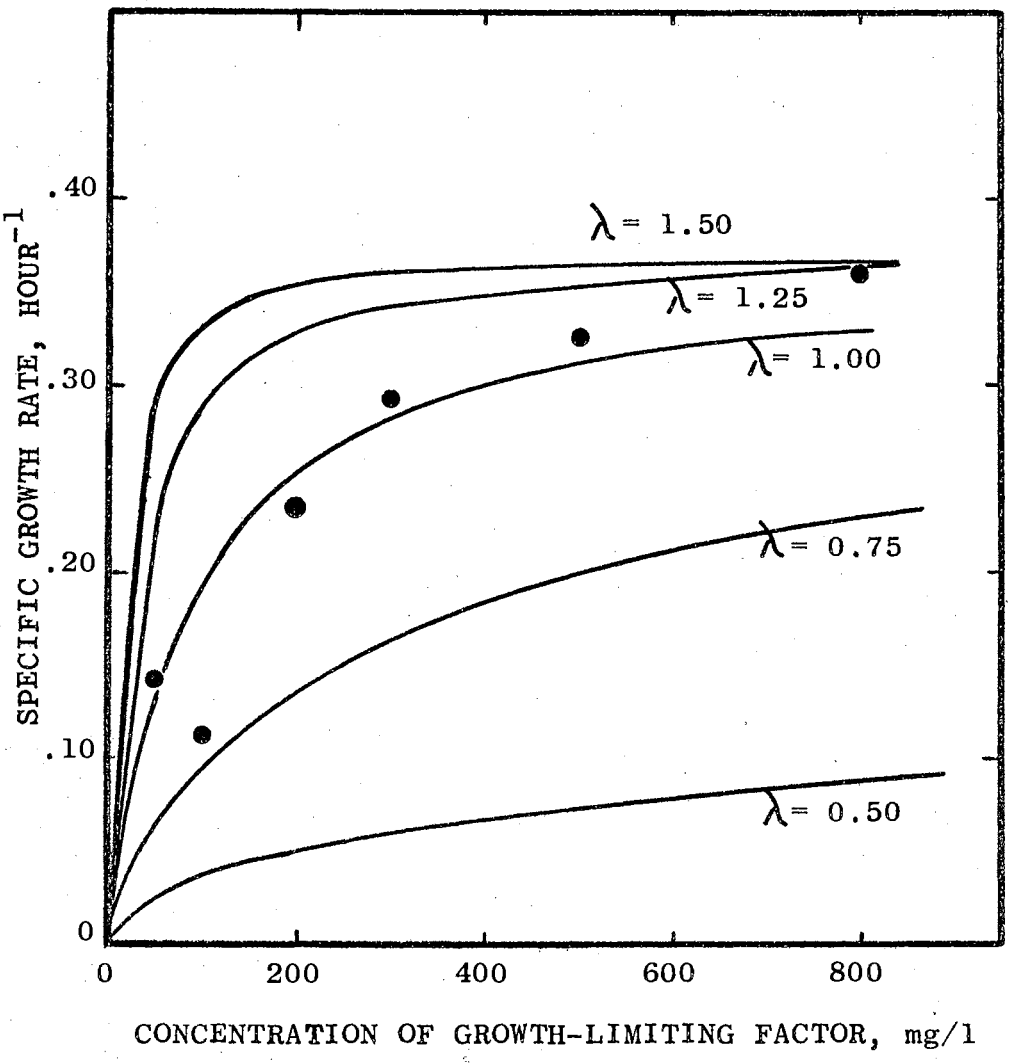

Figure 31. Relationship between μ and S according to the Theory of Moser. (S_i = 1000 mg/l Glucose; D = 1/18 Hour^{-1}; without Recirculation)

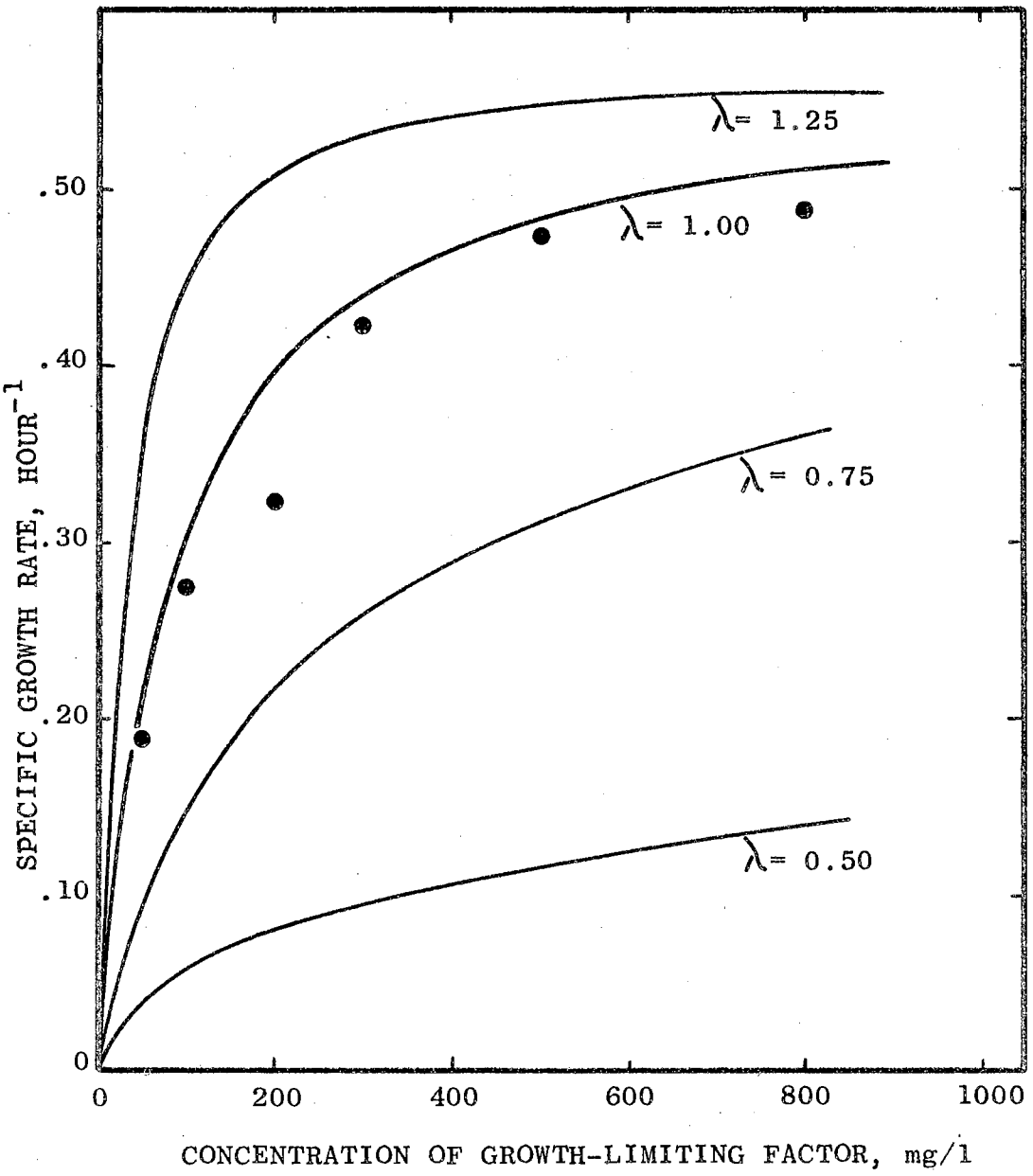

Figure 32. Relationship between μ and S according to the Theory of Moser at D = 1/12 Hour^{-1}. (S_i = 1000 mg/l Glucose; without Recirculation)

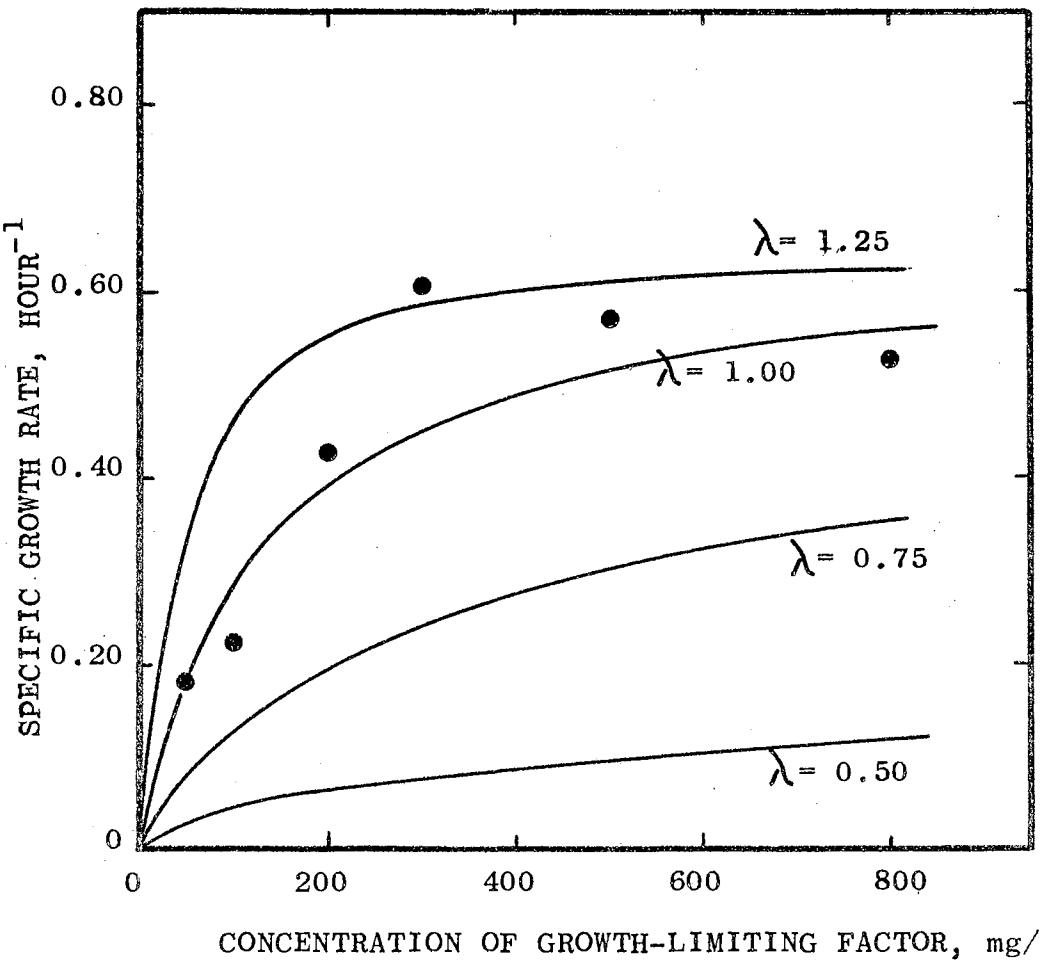

Figure 33. Relationship between μ and S according to the Theory of Moser at D = 1/6 Hour^{-1}. (S_i = 1000 mg/l Glucose; without Recirculation)

Table II. These curves represent the typical results obtained at all dilution rates.

In Figures 34 through 49 are shown the experimental results of growth rate studies using cells harvested from the steady-state units which were operated at different dilution rates with an inflow substrate concentration of 3000 mg/l without recirculation of sludge. Table III shows the values of the physiological growth parameters determined by batch experiments and evaluated according to the theory of Monod. Examination of the values in Table III confirms the earlier statement (see Table II) that the physiological parameters of growth are not really constants for heterogeneous population; they are seen to vary with the dilution rate. The value of μ_m increases with increaing value of flow rate or dilution rate up to a certain value, and then drops to lower values. The maximum values of μ_m attained were 0.69 and 0.64 hr^{-1} for studies without recirculation, and with S_i values of 1000 mg/l and 3000 mg/l glucose respectively. As in the studies at 1000 mg/l glucose, the variation in the values of k_s does not follow any definable pattern.

The fit of the experimental data to Equation 67 proposed by Teissier and adopted by Schulze (90) is shown in Figures 50 and 51. Equation 67 can be rewritten in the following manner:

$$-\frac{S}{k_s} = 2.3 \log \left(1 - \frac{\mu}{\mu_m}\right) \qquad (93)$$

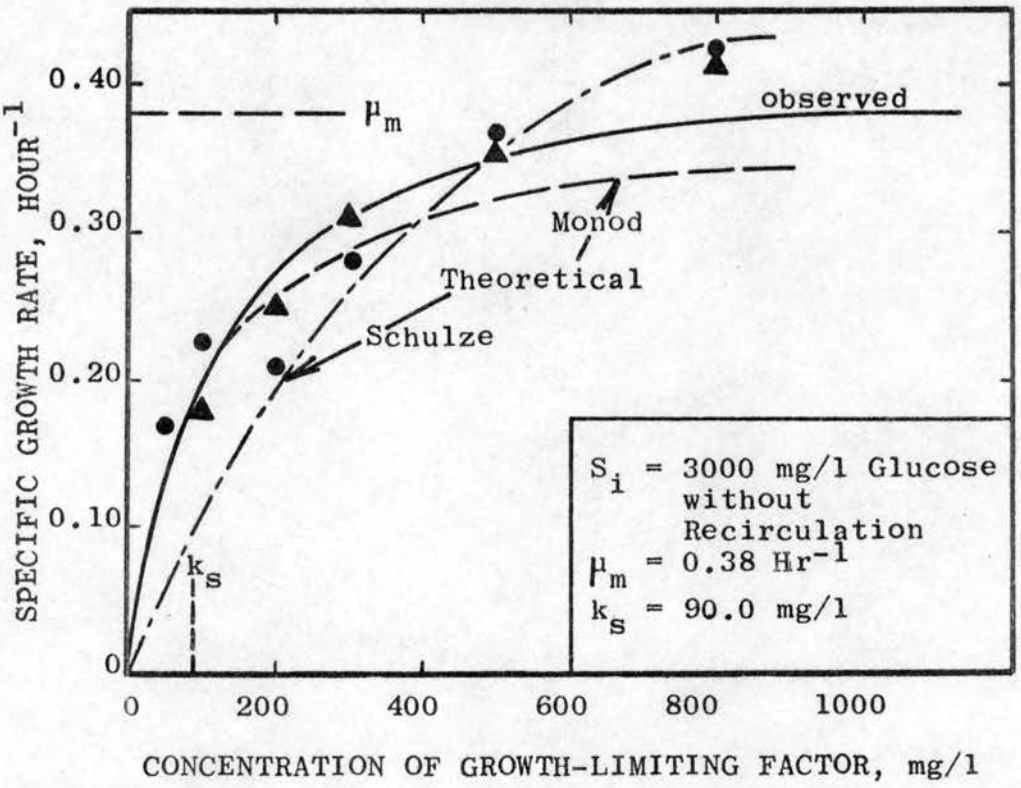

Figure 34. Relationship between μ and S at D = 1/18 Hour^{-1}.

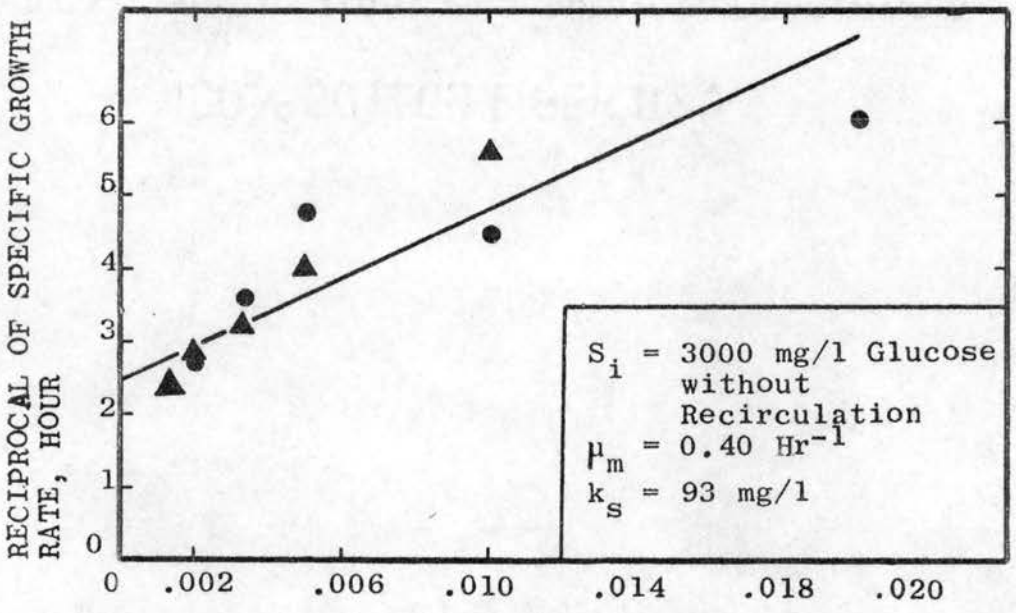

Figure 35. Relationship between $1/\mu$ and 1/S at D = 1/18 Hour^{-1}.

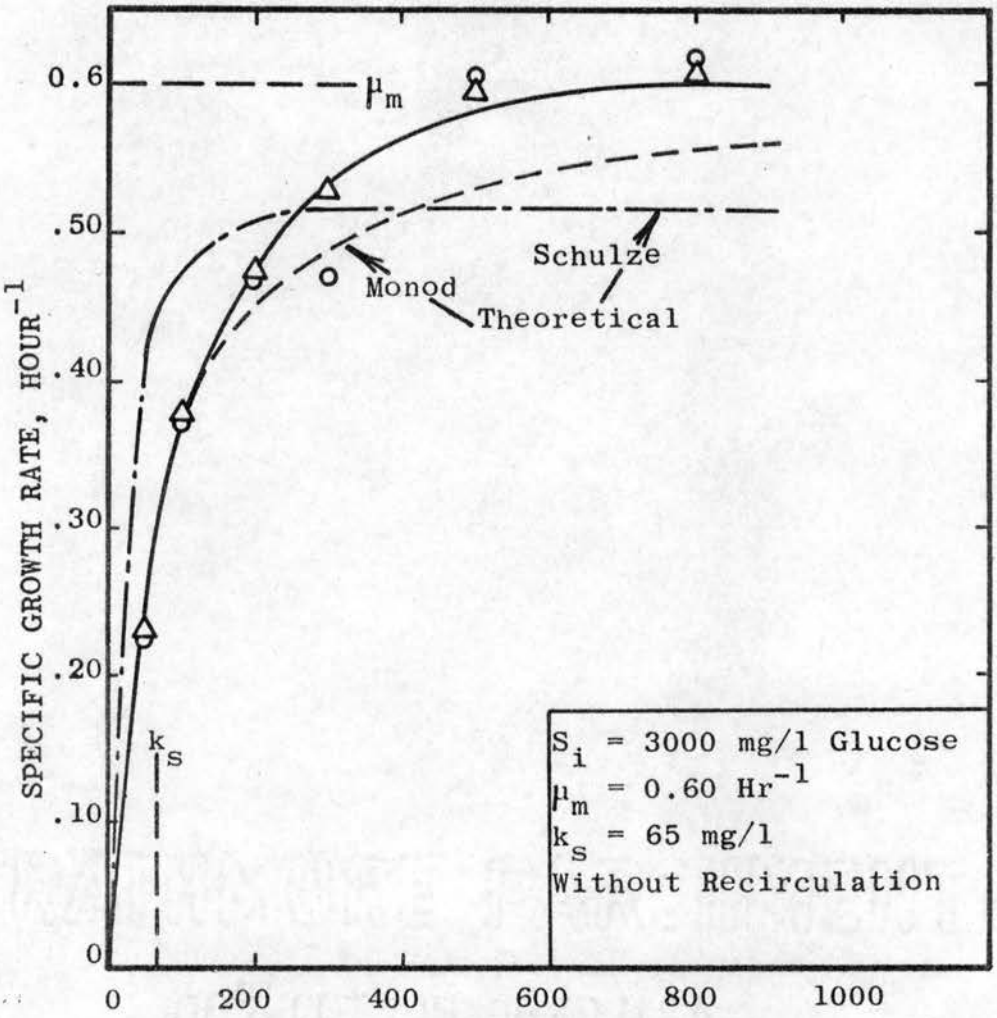

Figure 36. Relationship between μ and S at $D = 1/12\ Hour^{-1}$.

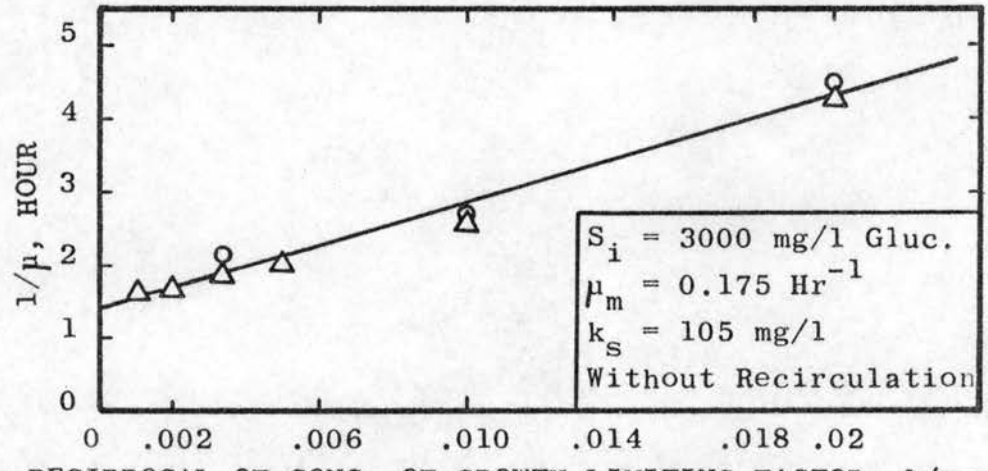

Figure 37. Relationship between $1/\mu$ and 1/S at $D = 1/12\ Hour^{-1}$.

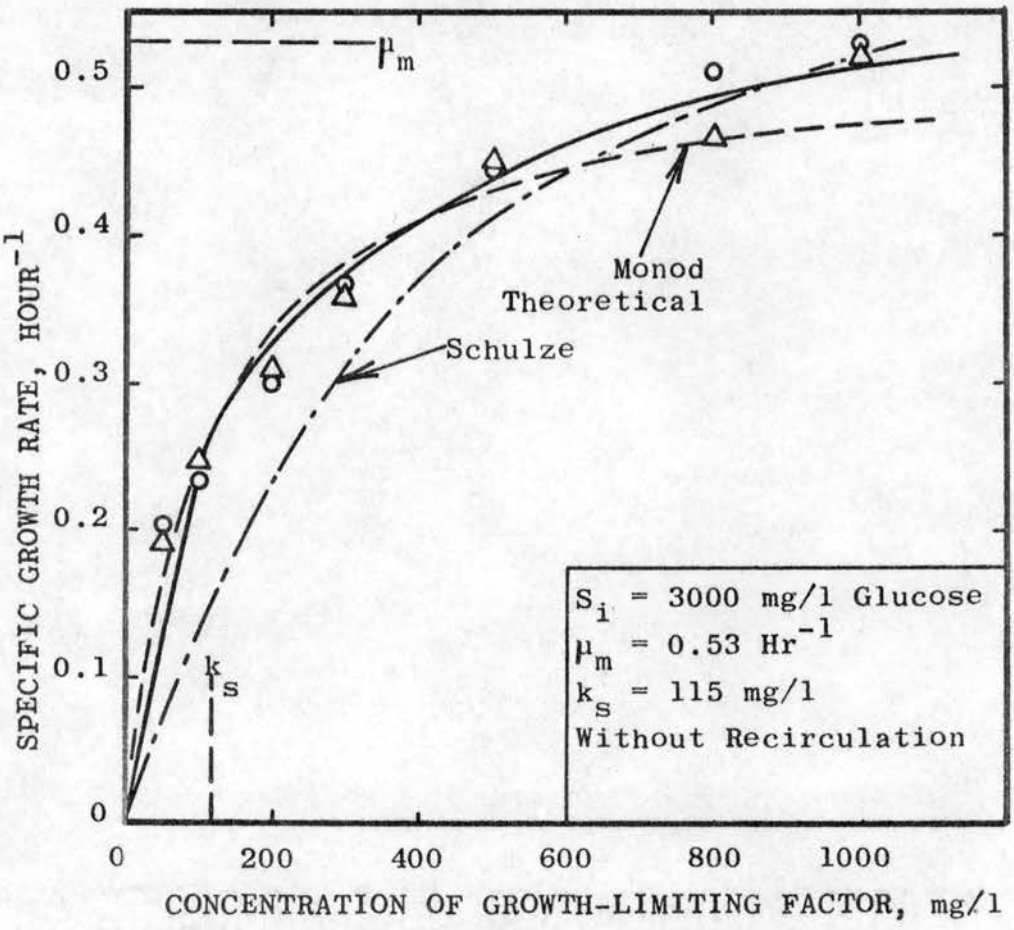

Figure 38. Relationship between μ and S at D = 1/6 Hour^{-1}.

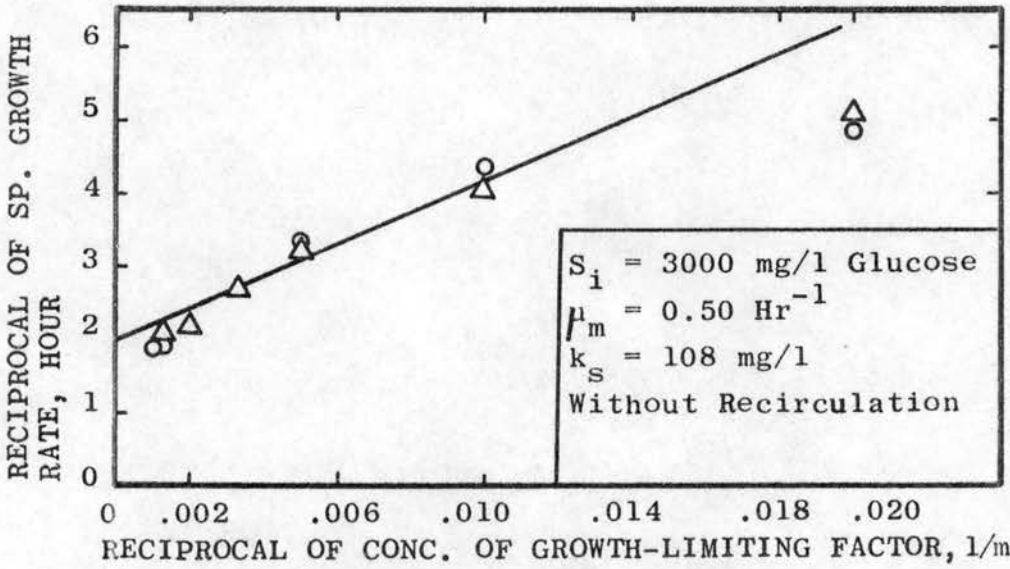

Figure 39. Relationship between $1/\mu$ and 1/S at D = 1/6 Hour^{-1}.

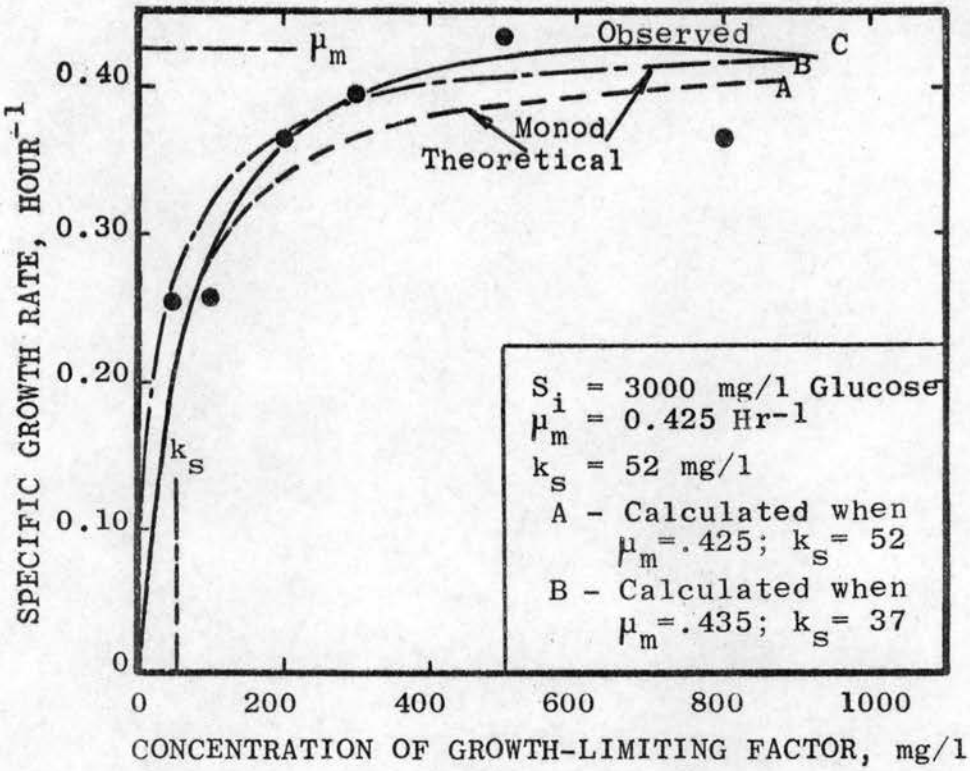

Figure 40. Relationship between μ and S at D = 1/24 $Hour^{-1}$.

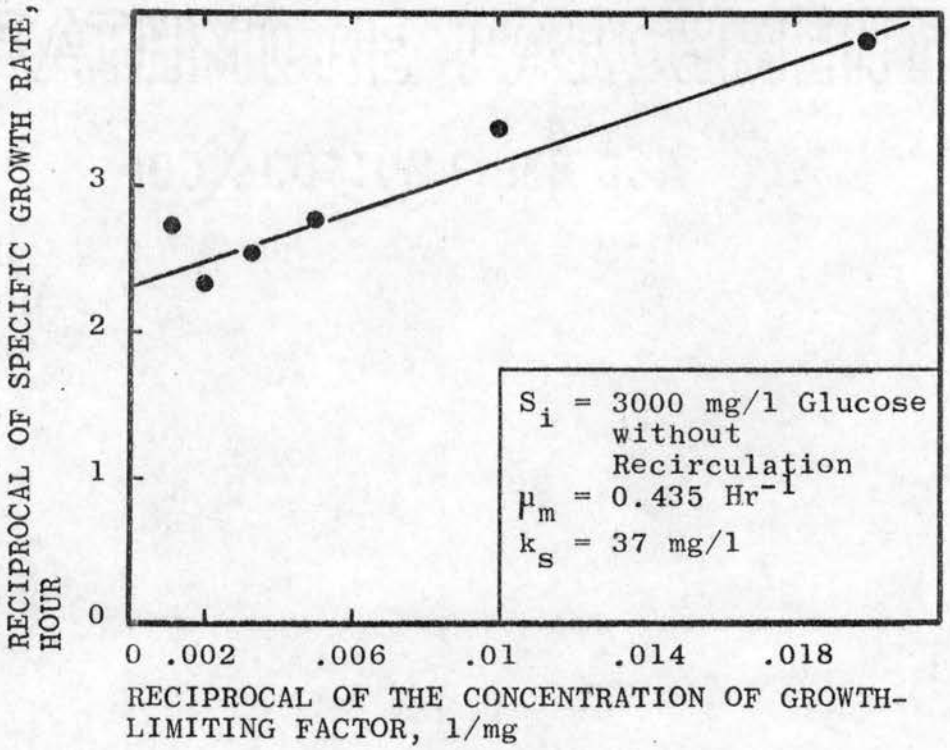

Figure 41. Relationship between $1/\mu$ and 1/S at D= 1/24 $Hour^{-1}$.

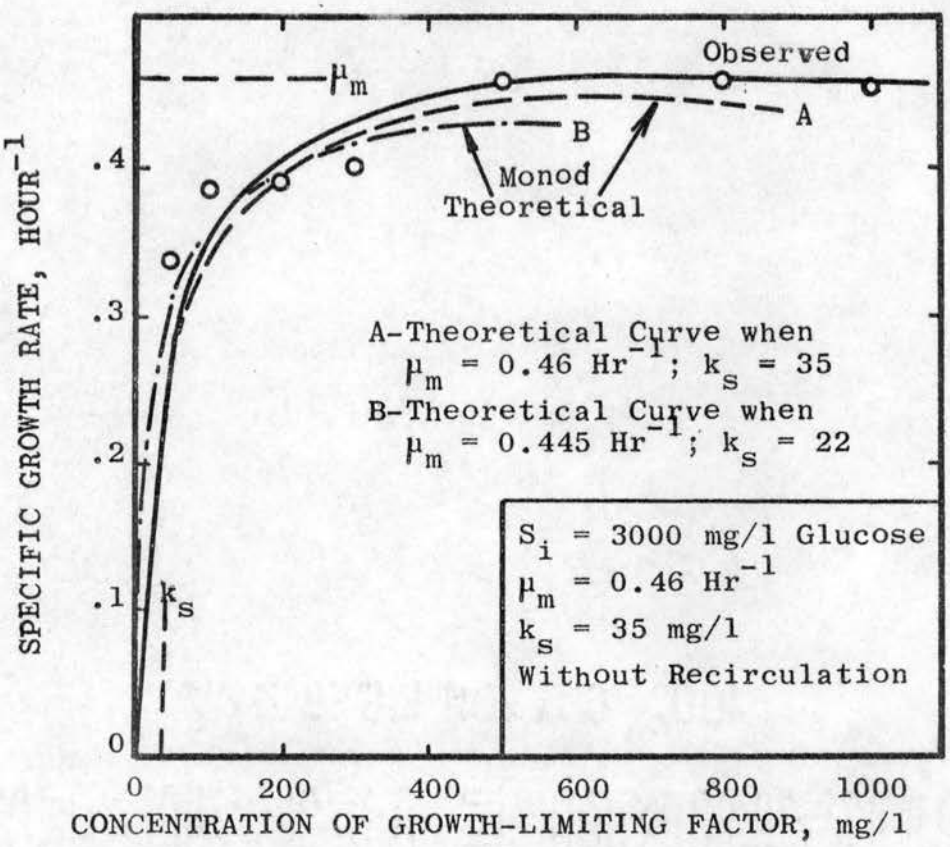

Figure 42. Relationship between μ and S at D = 1/3 Hour^{-1}.

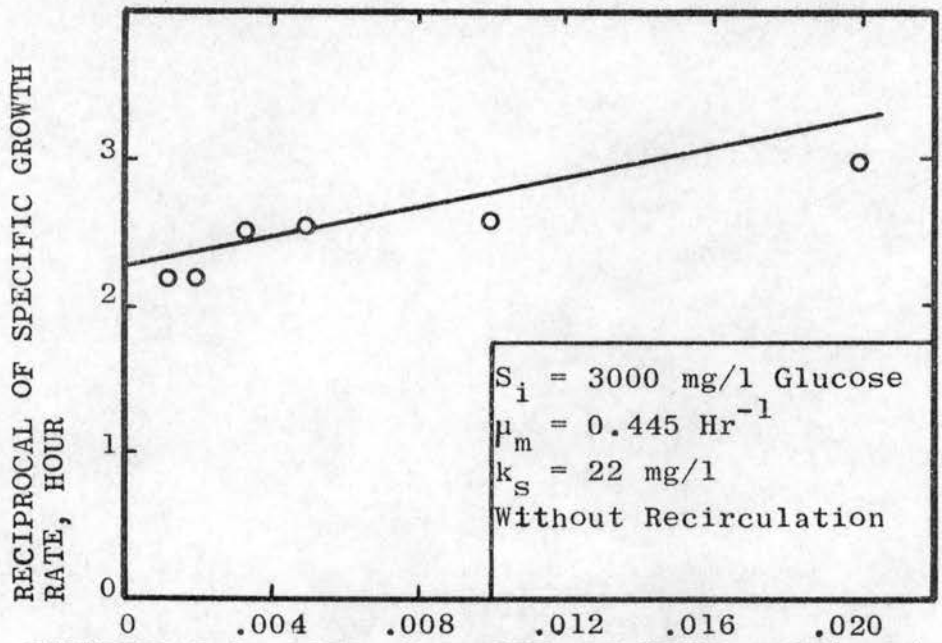

Figure 43. Relationship between $1/\mu$ and 1/S at D = 1/3 Hour^{-1}.

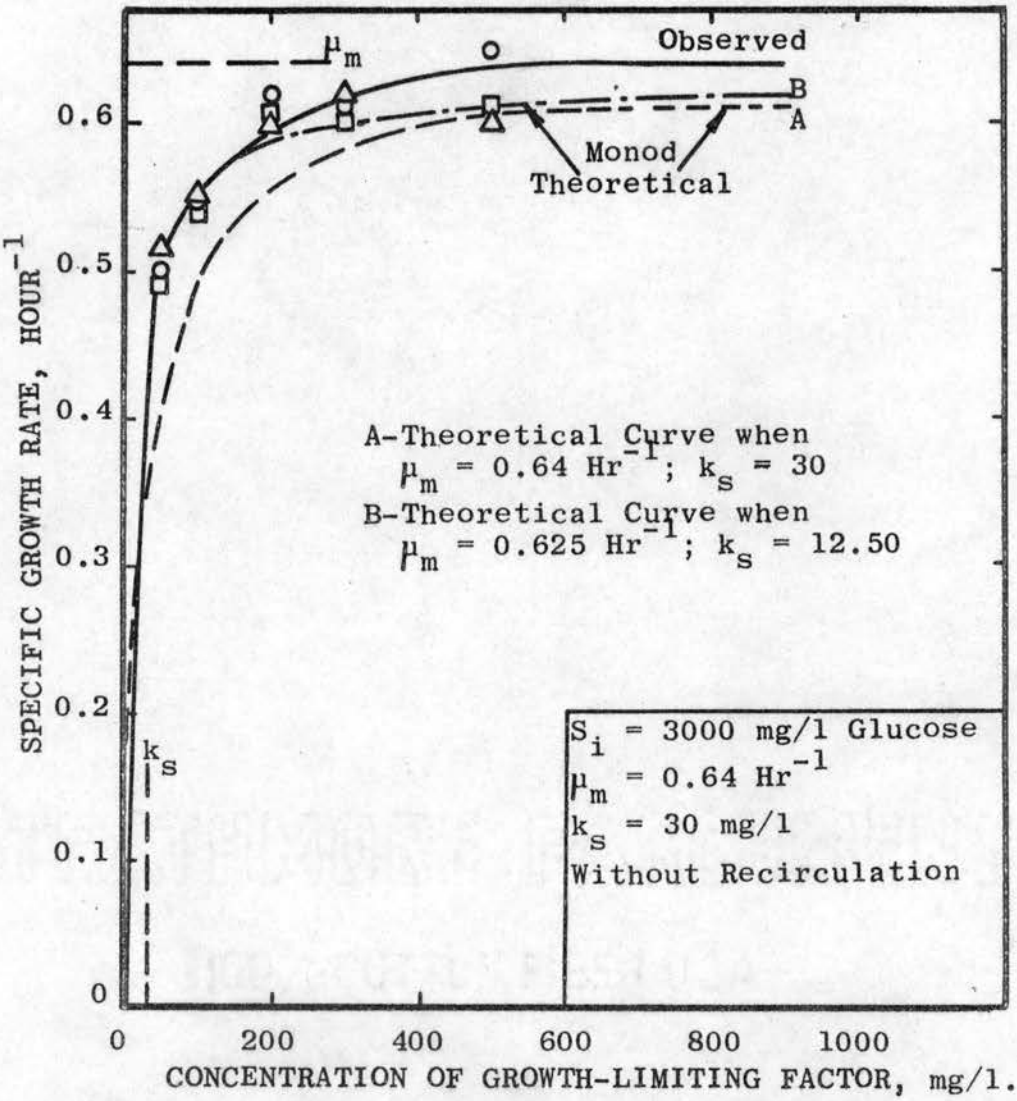

Figure 44. Relationship between μ and S at D = 1/2 Hour^{-1}.

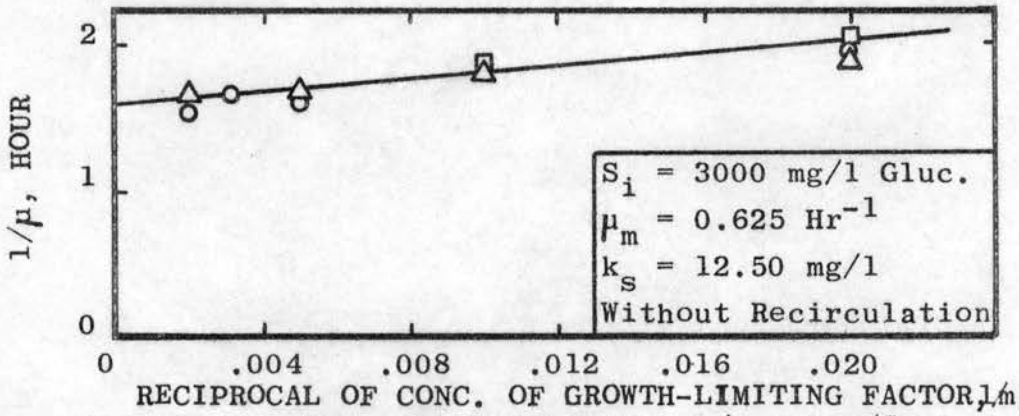

Figure 45. Relationship between $1/\mu$ and 1/S at D = 1/2 Hour^{-1}.

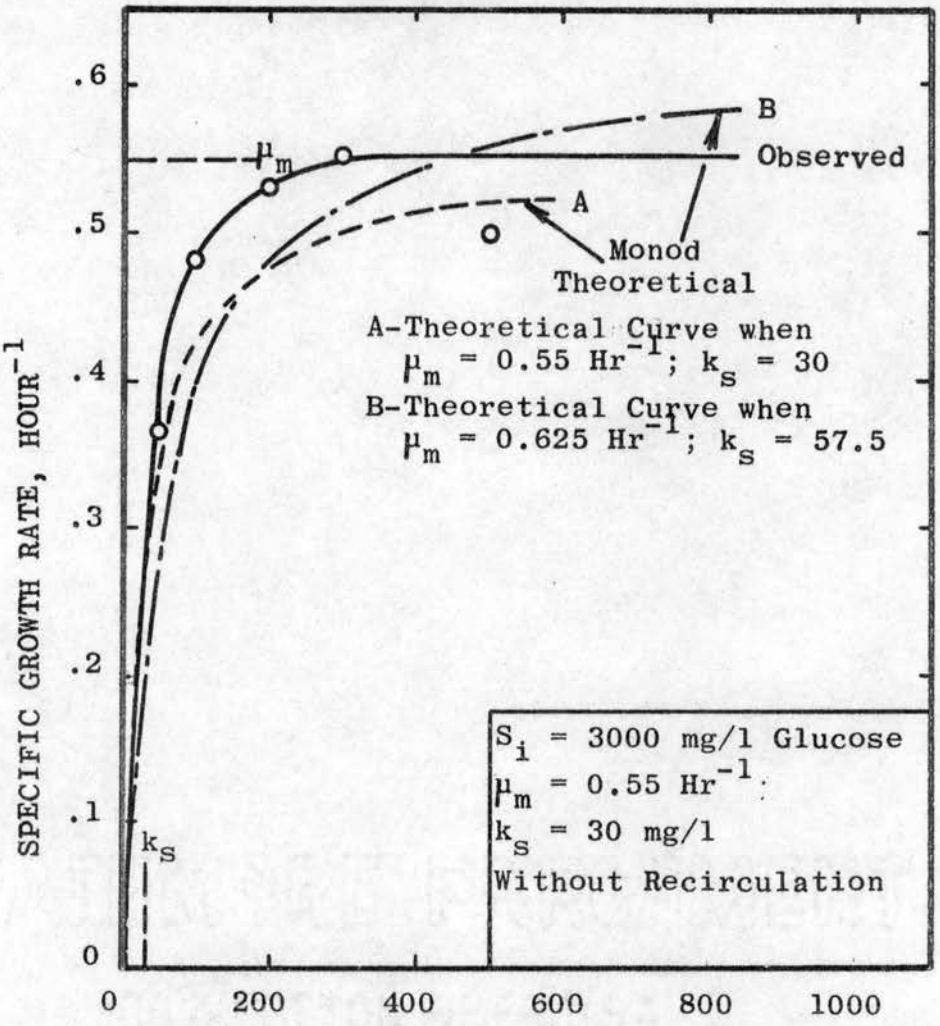

Figure 46. Relationship between μ and S at D = 1/1.50 Hour^{-1}.

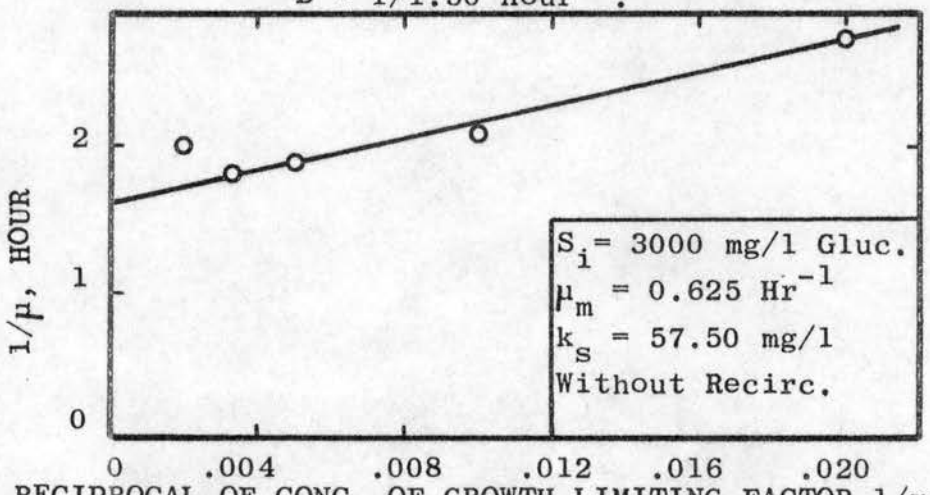

Figure 47. Relationship between $1/\mu$ and 1/S at D = 1/1.50 Hour^{-1}.

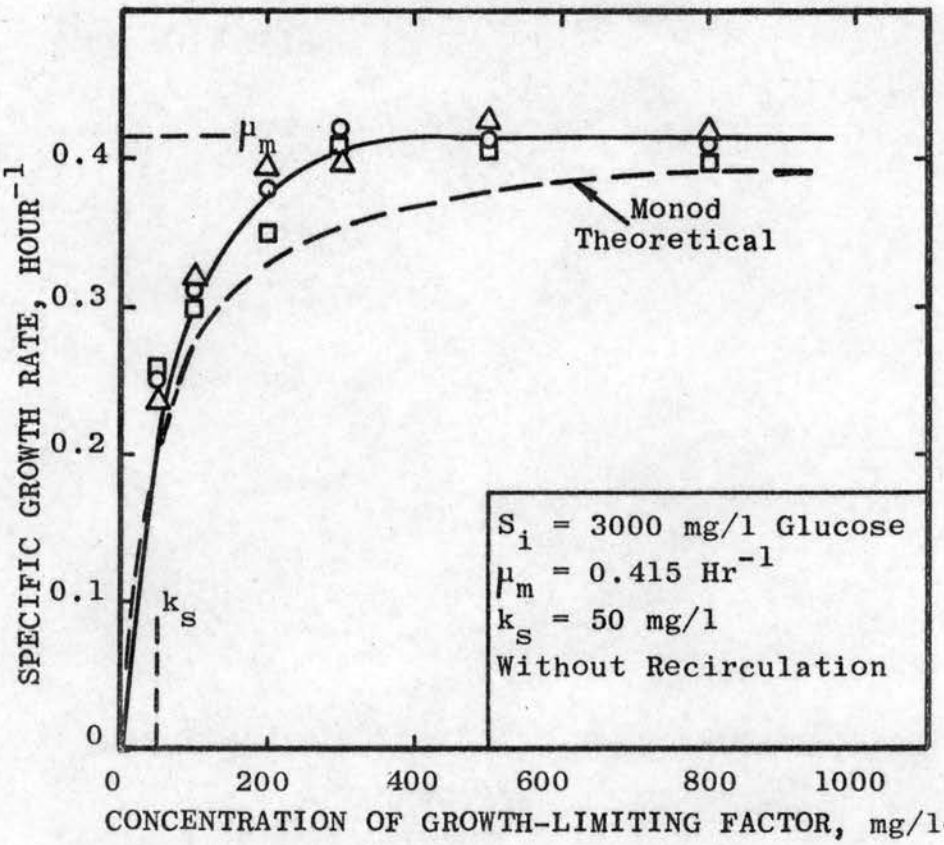

Figure 48. Relationship between μ and S at D = 1.0 Hour^{-1}.

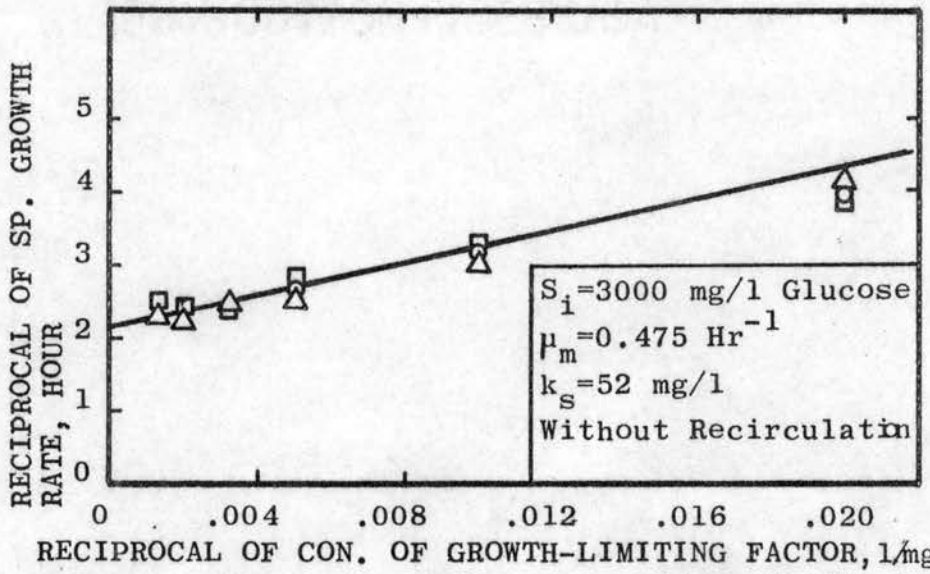

Figure 49. Relationship between $1/\mu$ and $1/S$ at D = 1.0 Hour^{-1}.

TABLE III

GROWTH PARAMETERS OBTAINED FROM BATCH EXPERIMENTS ACCORDING TO MONOD'S THEORY

(S_i = 3000 mg/l Glucose; No Recirculation)

Dilution Rate	Specific Growth Rate, Hr^{-1} Concentration of Substrate, mg/l						μ_{m1}	k_{s1}	μ_{m2}	k_{s2}	μ_m	k_s	Yield Coefficient (Y)	
	50	100	200	300	500	800	Hr^{-1}		Hr^{-1}		Hr^{-1}		Batch	Cont.
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
1/24	0.254	0.257	0.363	0.393	0.432	0.364	0.425	52	0.435	37	0.430	45	0.606	0.562
1/18	0.169	0.203	0.230	0.295	0.359	0.418	0.380	90	0.400	93	0.390	92	0.406	0.664
1/12	0.228	0.375	0.472	0.500	0.600	0.615	0.600	65	0.715	105	0.658	85	0.470	0.670
1/6	0.200	0.241	0.306	0.363	0.446	0.488	0.53	115	0.500	108	0.520	113	0.450	0.633
1/3	0.336	0.386	0.393	0.400	0.459	0.458	0.46	35	0.445	22	0.453	29	0.560	0.572
1/2	0.503	0.528	0.608	0.605	0.620	-	0.64	30	0.625	13	0.633	22	0.500	0.608
1/1.50	0.365	0.482	0.530	0.552	0.500	-	0.55	30	0.625	58	0.588	29	0.440	0.430
1	0.250	0.312	0.376	0.410	0.416	0.410	0.415	50	0.475	52	0.445	51	362	0.365

Note: μ_{m1} and k_{s1} are calculated from the graph μ vs. S

μ_{m2} and k_{s2} are calculated from the graph $1/\mu$ vs. $1/S$

μ_m and k_s are the mean values of μ_{m1} and μ_{m2}, and k_{s1} and k_{s2}

$$\text{or} \quad -S = k_s' \log (\mu_m - \mu) - k_s' \log \mu_m$$

$$\text{or} \quad \log (\mu_m - \mu) = \log \mu_m - \frac{S}{k_s'} \tag{94}$$

where $k_s' = 2.30\ k_s$

Equation 94 is in the form of a straight line, and a plot of log $(\mu_m - \mu)$ versus S should give a straight line. Figures 50 and 51 show such plots, and it can be seen that the observed data do not precisely fit a straight line. Figures 50 and 51 show the results for all experiments using inflow substrate concentrations of 1000 and 3000 mg/l respectively. Ahother method to test the applicability of Equation 67 has been presented by Schulze; Equation 67 can be written in a different form, as follows:

$$\frac{d\mu}{dS} = k_s (\mu_m - \mu) \tag{95}$$

Equation 95 can be rewritten as

$$\frac{\Delta\mu}{\Delta S} = k_s \mu_m - k_s \mu \tag{96}$$

and a plot of $\Delta\mu / \Delta S$ versus μ should give a straight line. Such a graph is shown in Figure 52 for growth rate studies using cells harvested from the steady-state units run at dilution rates of 1/18, 1/12, and 1/6 per hour with an inflow substrate concentration of 3000 mg/l glucose. The data for plotting Figures 51 and 52 are taken from Table III. From Figure 52 it can be observed that the growth data obtained with the cultures which previously had been developed at 18-hour and 6-hour mean residence times

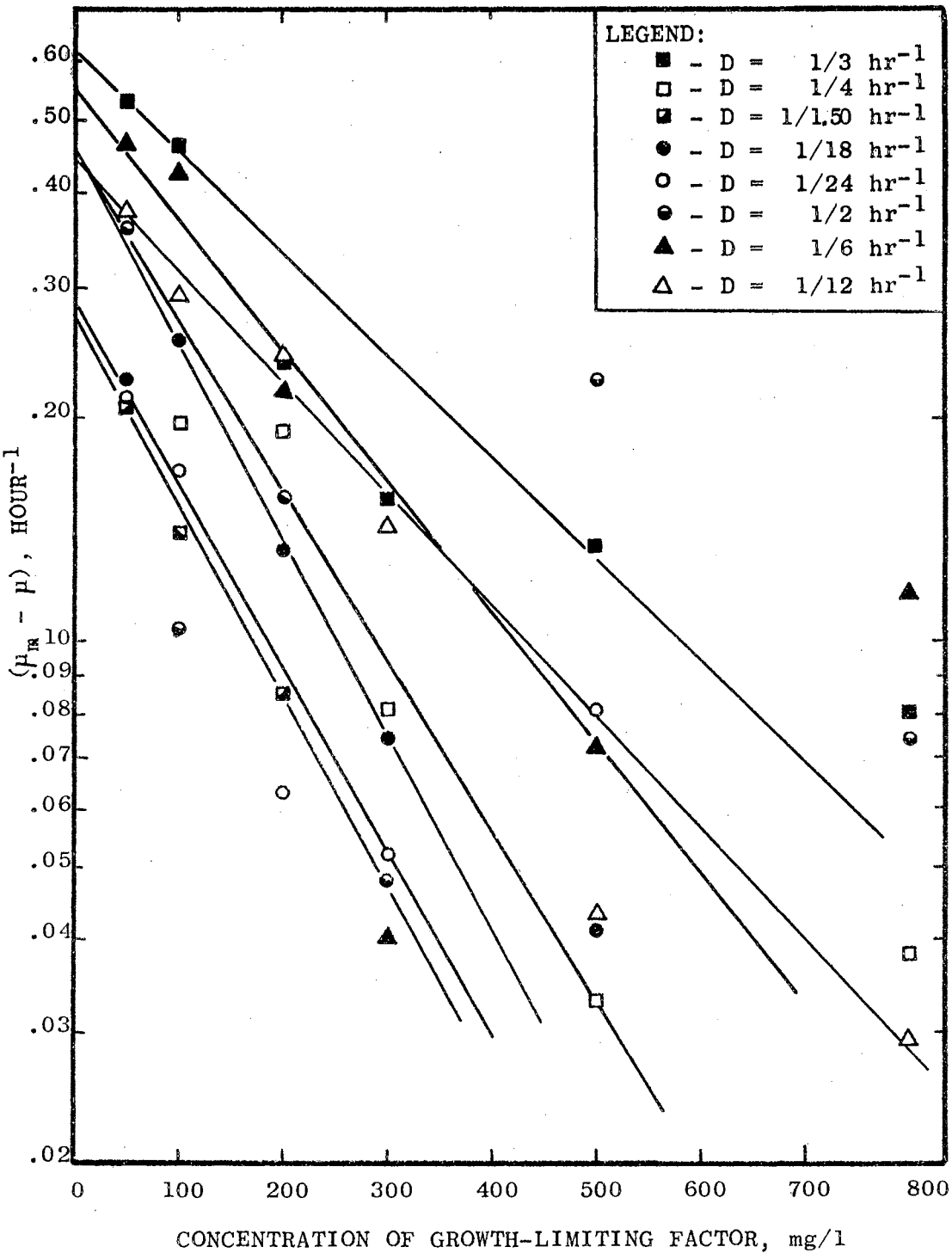

Figure 50. Verification of Equation 67 with the observed data obtained from Batch Experiments when S_i = 1000 mg/l Glucose.

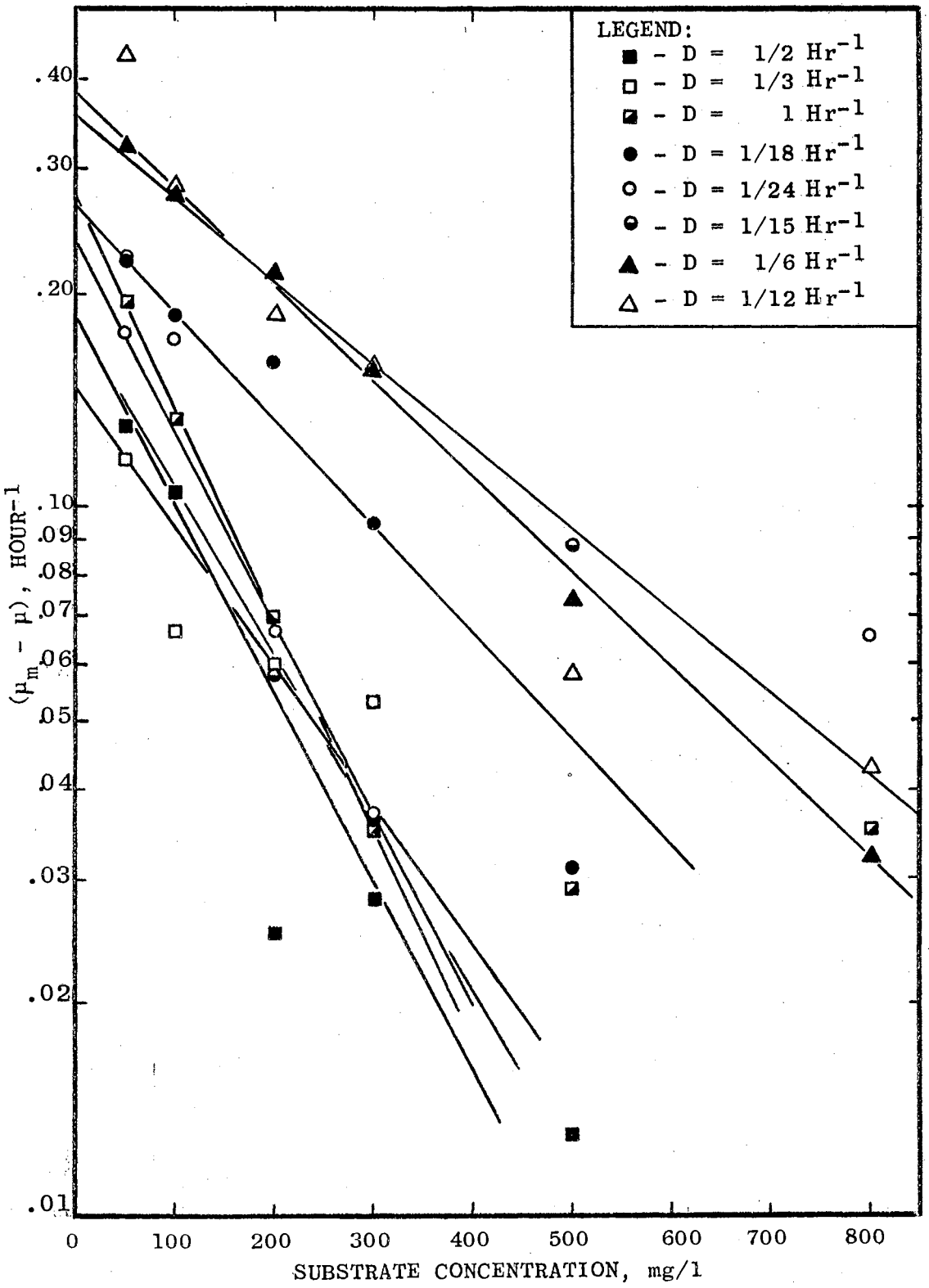

Figure 51. Verification of Equation 67 with the observed Data obtained from Batch Experiments when S_i = 3000 mg/l Glucose.

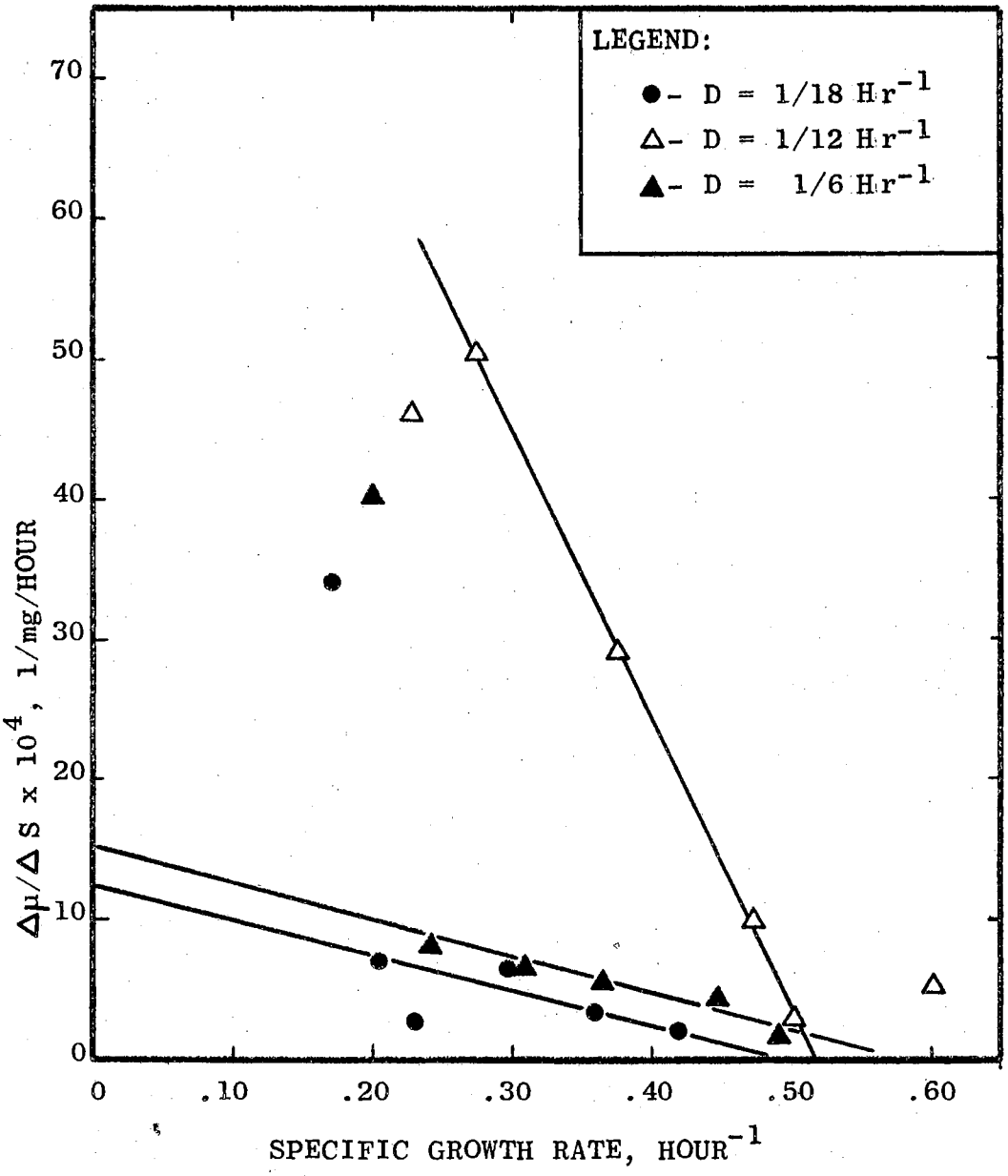

Figure 52. Relationship between $\Delta\mu/\Delta S$ and μ according to the Method of Schulze. (S_i = 3000 mg/l Glucose; without Recirculation)

give a better relationship than that of 12-hour mean residence time. In general, the data in all three cases are considerably scattered. From the intercepts of the lines with the ordinate and from the slopes of the lines, the values of k_s and μ_m were estimated and used to calculate the values of μ at different substrate concentrations employing Equation 67. For comparative purposes, these values are plotted along with experimental growth rate values and with the theoretical curve according to the expression proposed by Monod in Figures 34, 36, and 38. It is evident from these figures that the experimental growth rate values in the present study do not fit Schulze's expression. Further, it can be seen that Monod's theoretical curve fits more closely with the experimental values than that proposed by Schulze.

(b) Studies Employing Recirculation of Sludge

In Figures 53 to 60 are shown the relationships between substrate concentration and specific growth rate for cultures obtained from steady-state units operated with an inflow substrate concentration of 1000 mg/l glucose with recirculation of sludge. The recirculation factor employed was 0.25 of the inflow medium. It can be seen from the graphs that at all dilution rates studied, the experimental data fit fairly well with the values obtained according to the equation of Monod. Comparison of these data with the expressions of other investigators was not made,

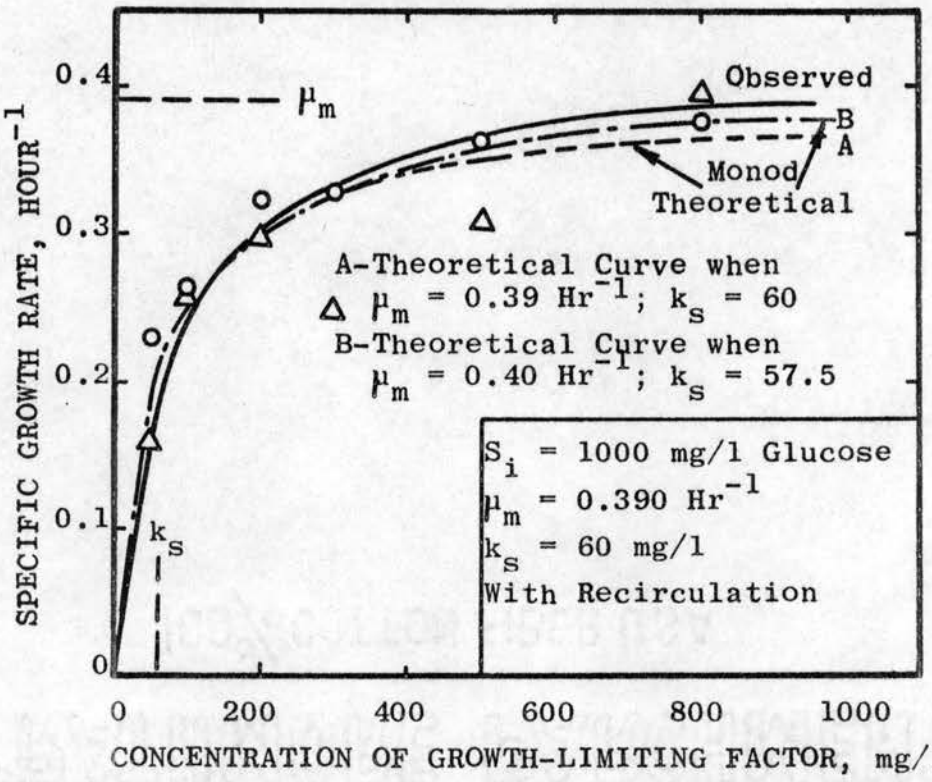

Figure 53. Relationship between μ and S at $\bar{t}$ = 6 Hours.

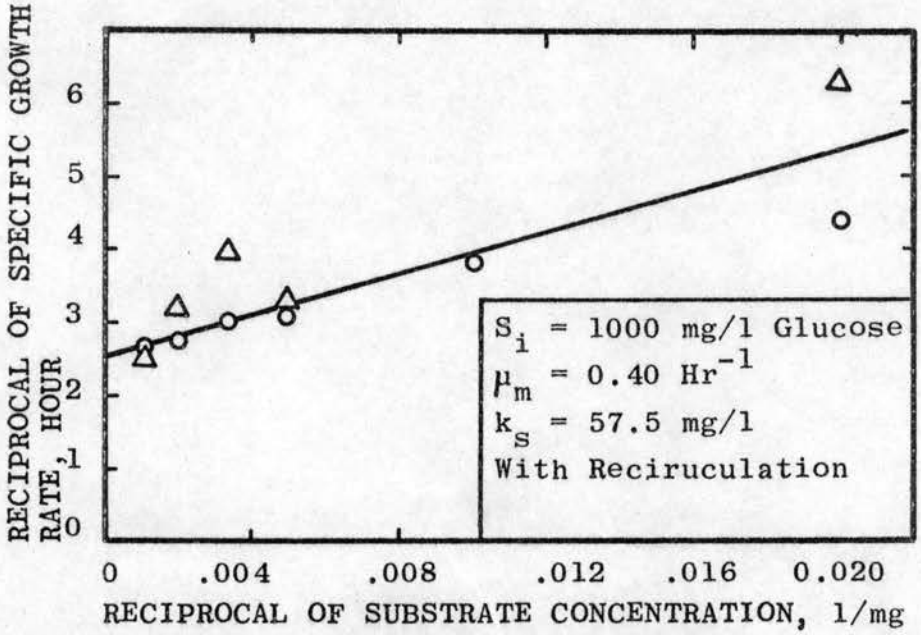

Figure 54. Relationship between $1/\mu$ and $1/S$ at $\bar{t}$ = 6 Hours

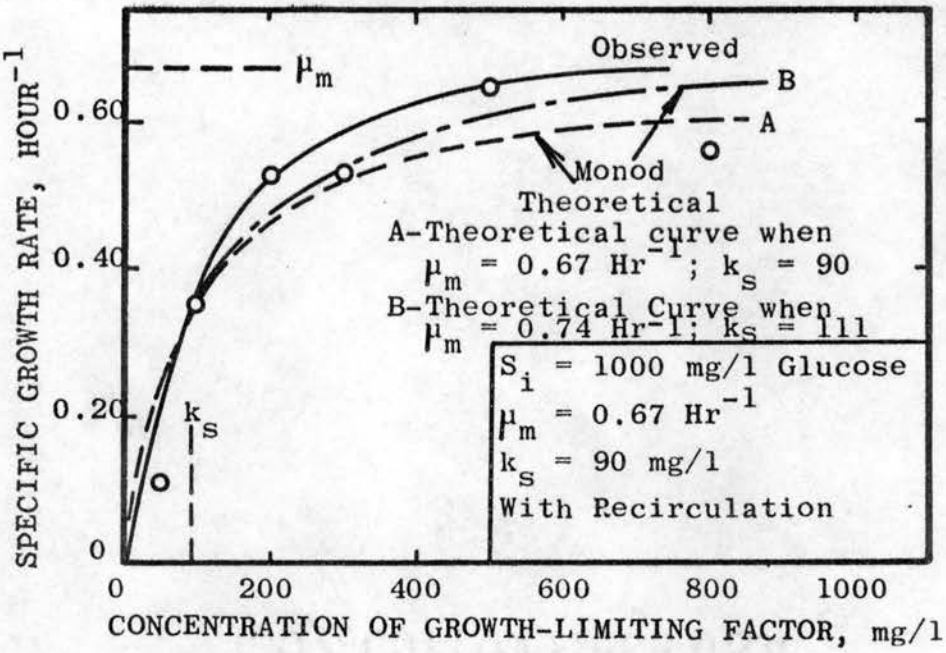

Figure 55. Relationship between μ and S at $\bar{t}$ = 4 Hours.

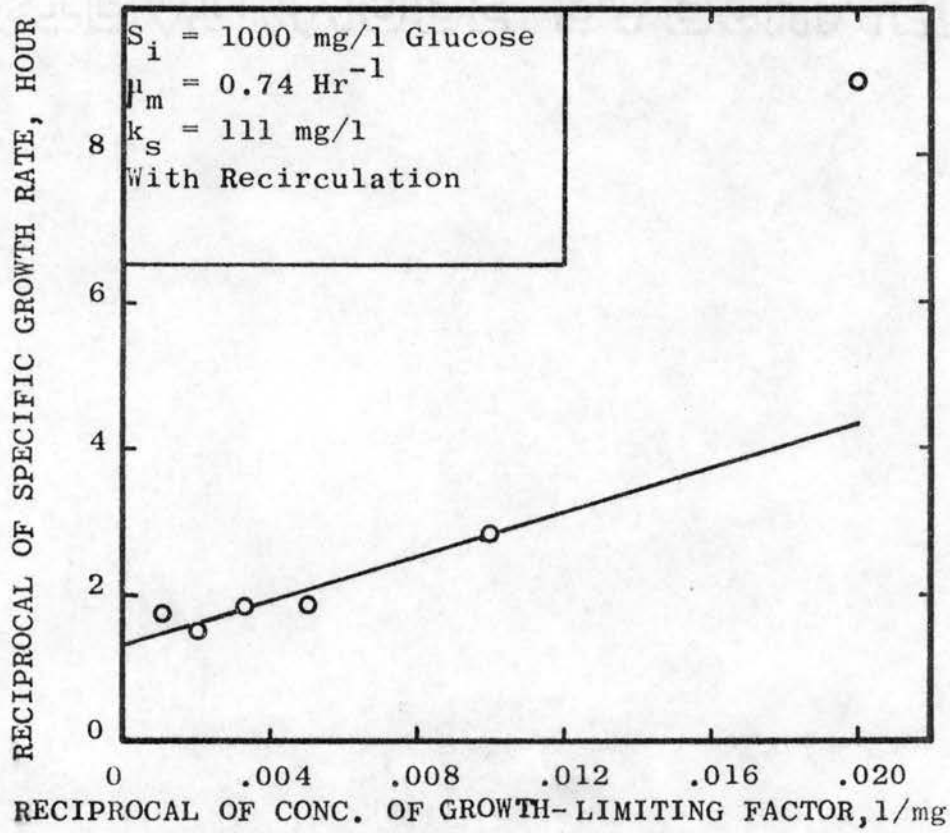

Figure 56. Relationship between $1/\mu$ and $1/S$ at $\bar{t}$ = 4 Hours.

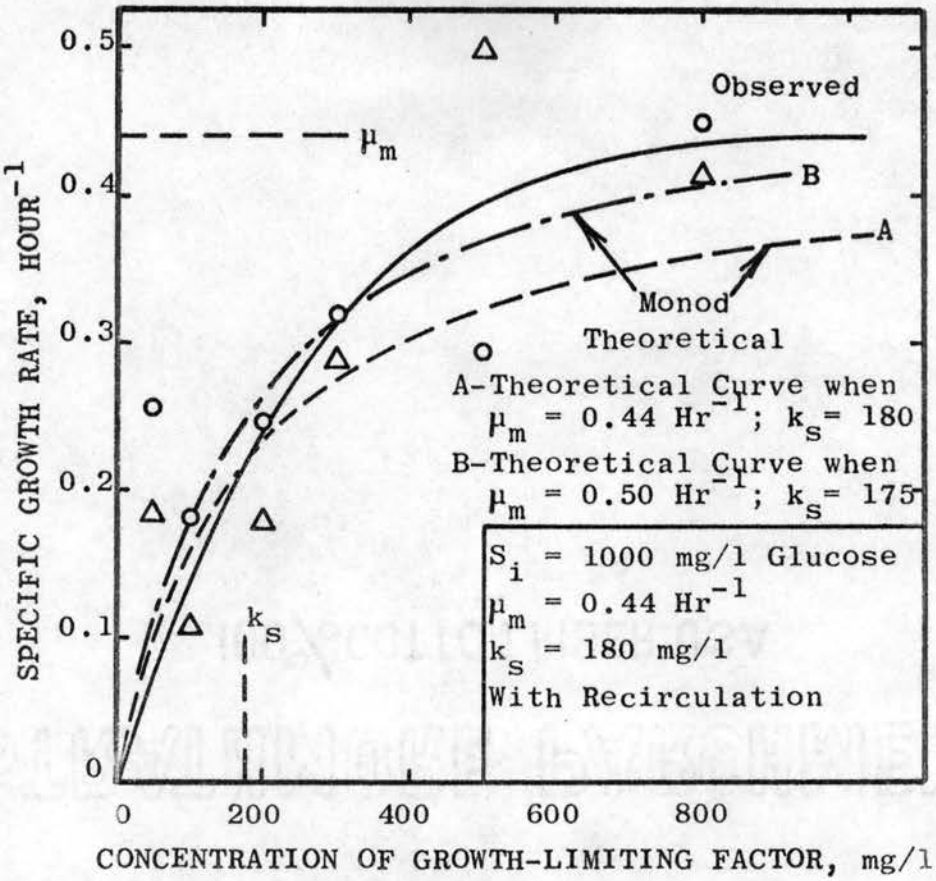

Figure 57. Relationship between μ and S at $\bar{t}$ = 3 Hours.

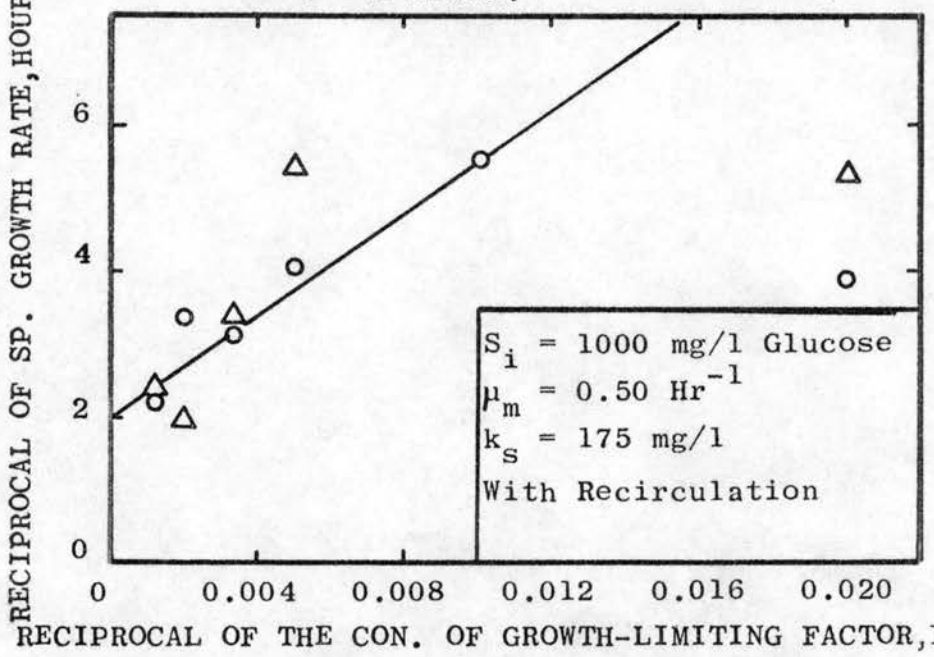

Figure 58. Relationship between $1/\mu$ and $1/S$ at $\bar{t}$ = 3 Hours.

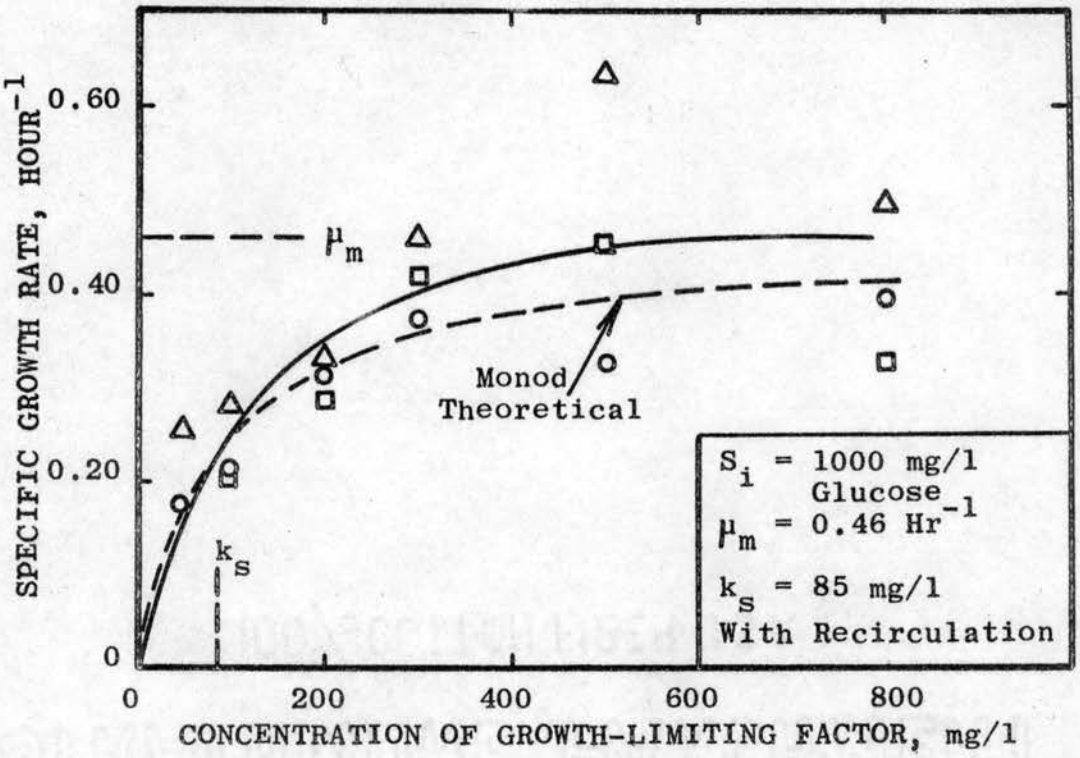

Figure 59. Relationship between μ and S at $\bar{t}$ = 2 Hours.

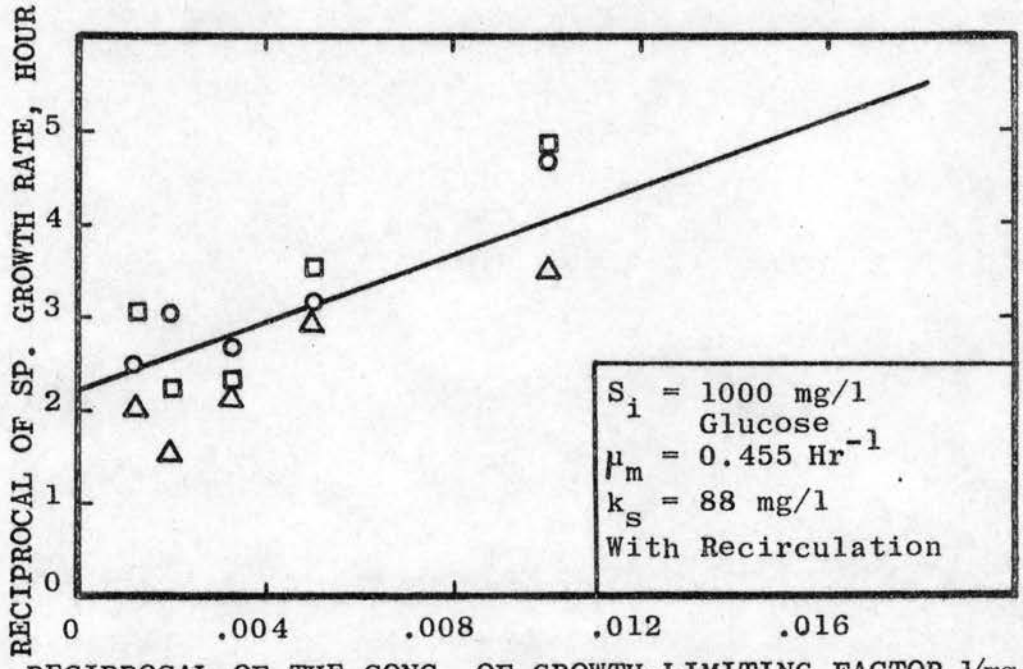

Figure 60. Relationship between $1/\mu$ and $1/S$ at $\bar{t}$ = 2 Hours.

since it was already shown that the other equations did not fit the experimental data for systems involving no sludge recycling. Table IV shows the values of the physiological growth parameters obtained at different dilution rates. It is apparent from the table that the values of the physiological parameters are similar to those obtained for experiments without recirculation of sludge (compare Tables II and III with IV).

Figures 61 and 62 show the relationship between substrate concentration and growth rate for the sludge harvested from units operated without sludge wasting. It can be seen that the experimental values follow a pattern similar to those presented previously. However, a difference in the magnitude of μ_m was observed. The μ_m value for this sludge was found to be 0.135 per hour, which is comparatively lower than the values for sludge taken from any of the other continuous-flow activated sludge units. This indicates that the sludge developed under closed system probably consists of predominantly slower-growing organisms than in open systems. Since the growth rates measured represent only the mean rates of growth of the individual species present in the system, the fraction of slower-growing organisms has a direct influence on the mean rate of growth. The yield coefficient for the sludge developed without sludge wasting has a value of 0.422, which is comparable with the other yield values.

TABLE IV

GROWTH PARAMETERS OBTAINED FROM BATCH EXPERIMENTS ACCORDING TO MONOD'S THEORY

(S_i = 1000 mg/l Glucose; With Recirculation)

Dilution Rate D	Specific Growth Rate, Hr^{-1} — Concentration of Substrate, mg/l						μ_{m1}	k_{s1}	μ_{m2}	k_{s2}	μ_m	k_s	Y_B
Hr^{-1}	50	100	200	300	500	800	Hr^{-1}	mg/l					
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
1/6	0.1950	0.2623	0.312	0.289	0.337	0.387	0.39	60	0.40	58	0.39	59	0.375
1/4	0.111	0.354	0.53	0.532	0.648	0.56	0.67	90	0.74	111	0.70	100	0.584
1/3	0.221	0.146	0.216	0.306	0.398	0.433	0.44	180	0.50	175	0.47	178	0.589
1/2	0.202	0.2353	0.311	0.421	0.470	0.407	0.46	85	0.46	85	0.46	85	0.440

Note: μ_{m1} and k_{s1} are calculated from the graph μ vs. S
μ_{m2} and k_{s2} are calculated from the graph $1/\mu$ vs. $1/S$
μ_m and k_s are the mean values of μ_{m1} and μ_{m2}, and k_{s1} and k_{s2}

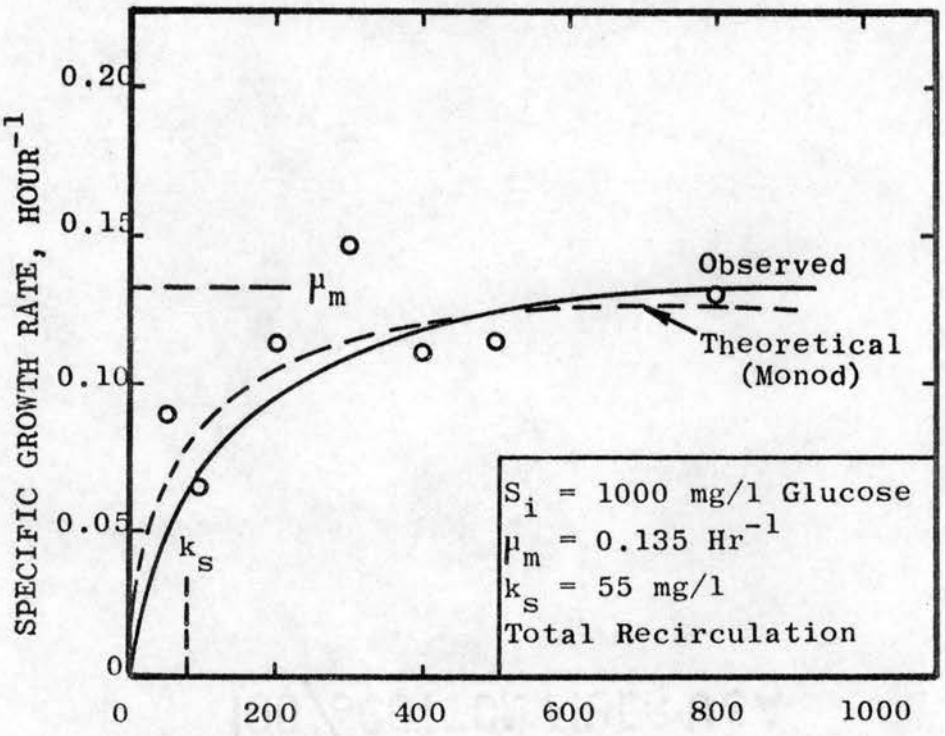

Figure 61. Relationship between μ and S at $\bar{t}$ = 4 Hours.

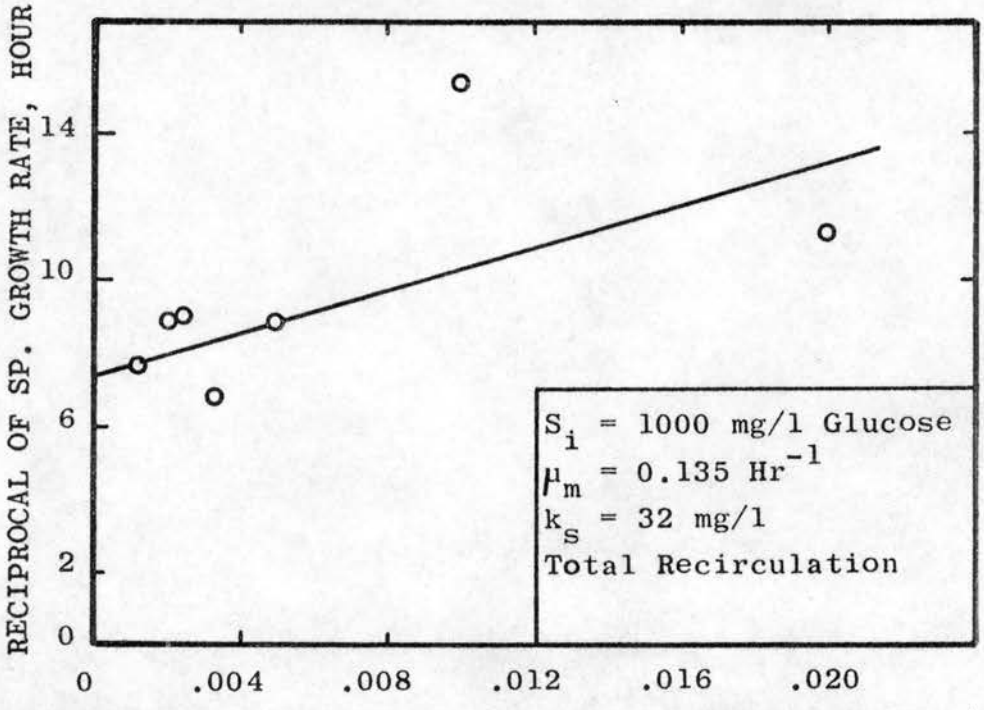

Figure 62. Relationship between $1/\mu$ and 1/S at $\bar{t}$ = 4 Hours.

2. Relationship between Oxygen Uptake Rate and Rate of Growth

Pirt (10) has shown that the rate of oxygen uptake could be related to rate of growth by Equation 45:

$$\frac{do}{dt} = \frac{p}{Y} \frac{dx}{dt} \qquad (45)$$

Monod (20) has also indicated that any metabolic activity associated with growth can be used as a measure of rate of growth. If such a relationship could be established, then the rate of growth might be determined more accurately without the pitfalls and complications inherent in the use of optical density as a measure of growth. Determination of growth by the optical density method has definite disadvantages, since flocculation of cells and colors introduced into the medium by bacterial metabolism or release of pigments can interfere with the optical density measurement. In order to study the feasibility of one such method, the rates of oxygen uptake and growth were plotted for a culture harvested from a continuous flow unit maintained in the steady state at a dilution rate of 0.667 per hour with an inflow substrate concentration of 3000 mg/l. Figure 63 shows the variations in the rates of growth and oxygen uptake with time. It can be seen from the graph that both curves follow the same pattern. Figure 64 shows the relationship between the rates of oxygen uptake and growth. It is apparent from the figure that they are related by a straight line which can be described by the

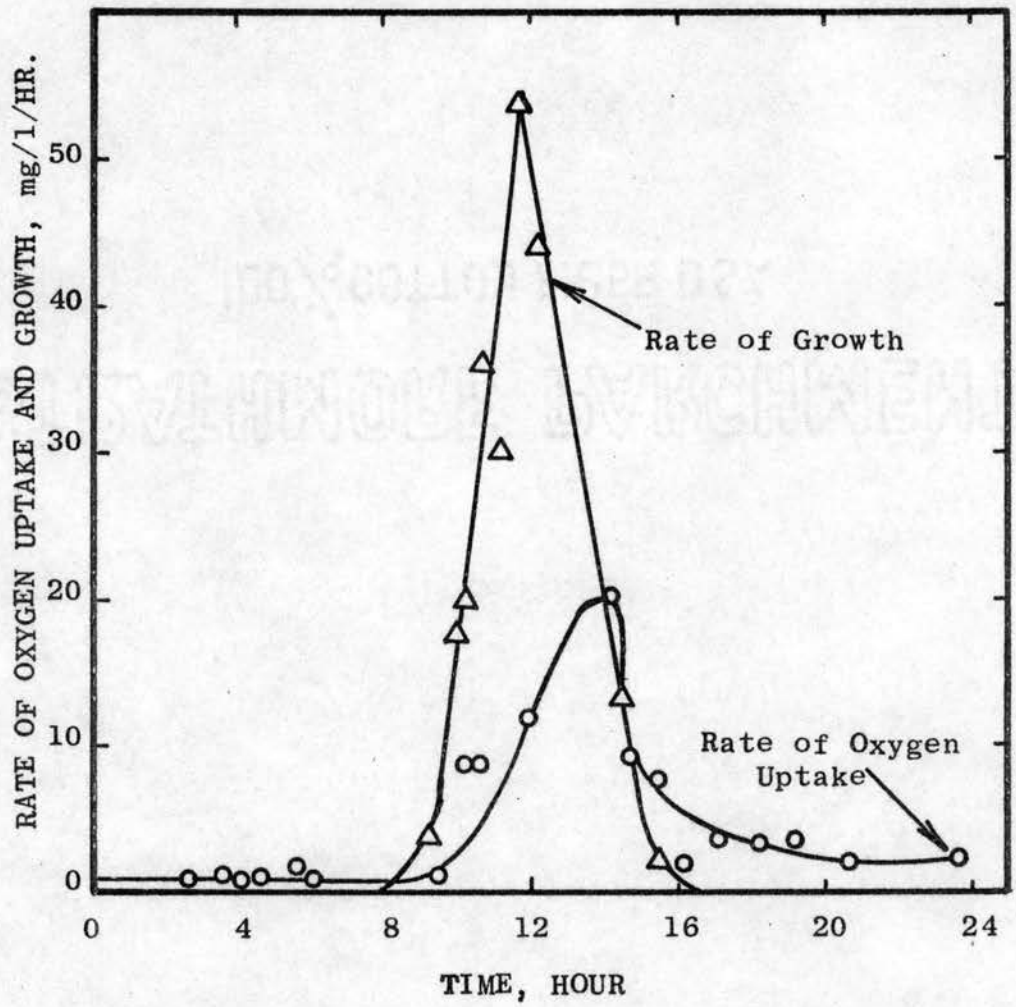

Figure 63. Rate of Growth and Rate of Oxygen Uptake at D = 1/1.50 Hour^{-1}, with S_i = 3000 mg/l Glucose.

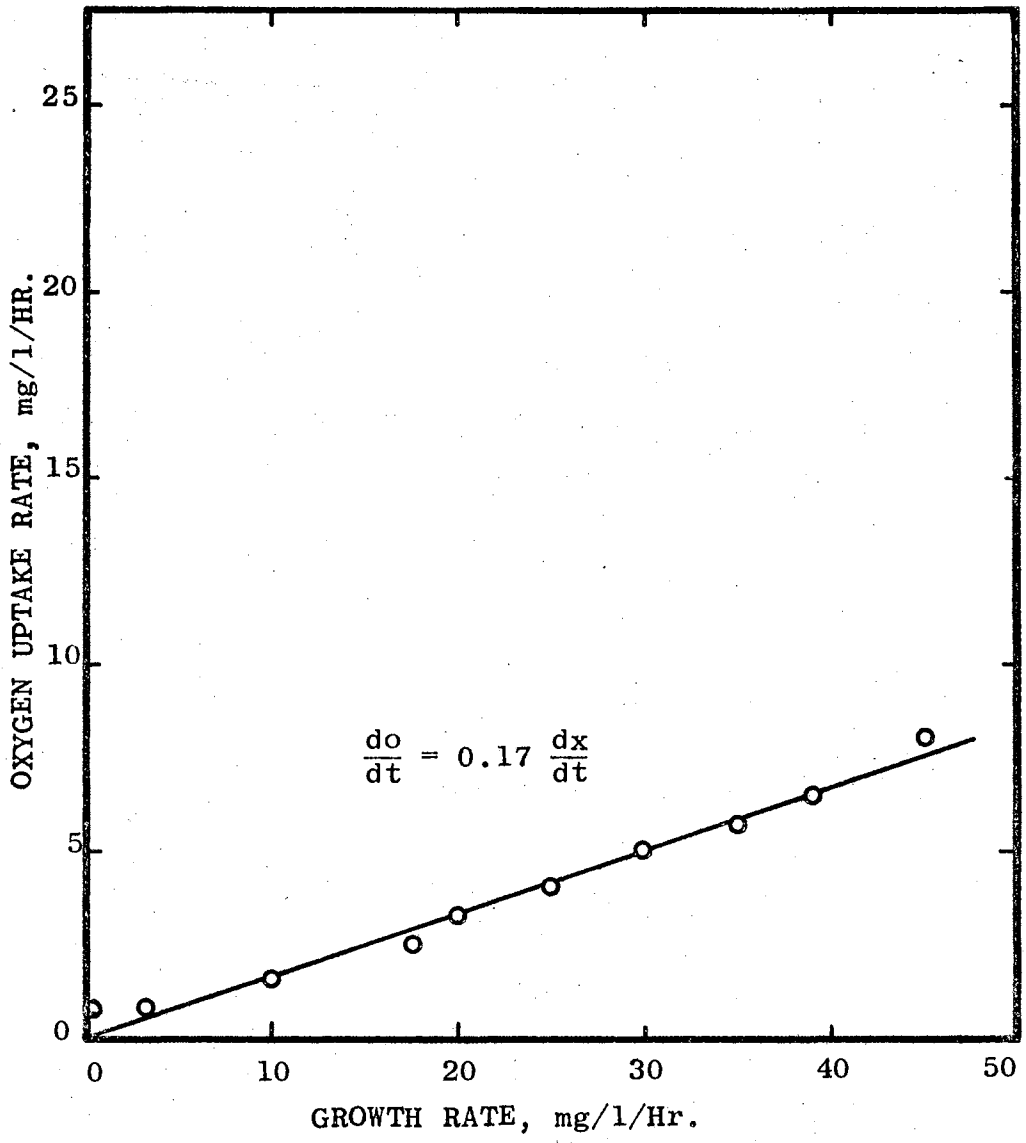

Figure 64. Relationship between Rate of Oxygen Uptake and Growth.

following equation:

$$\frac{do}{dt} = 0.17 \frac{dx}{dt} \tag{97}$$

Since this is based on only one experiment, further evidence is required before this method could be applied for growth rate determinations with heterogeneous populations.

Further evidence of the relationship between oxygen uptake rate and rate of growth was presented in Figures 28 and 29. Figure 28 shows the relationship between substrate concentration and exponential rate of oxygen uptake, together with the exponential growth rate. In Figure 29 the same data are shown on a reciprocal plot. It is evident from these two figures that the exponential rate of oxygen uptake and the exponential growth rate are similarly related to the concentration of growth-limiting factor.

3. Relationship between Growth and Substrate Consumption

The relationship between growth and substrate is an important parameter for establishing the kinetic relationships as well as for estimating the amount of sludge production. Examination of the yield values in Tables II to IV indicates that yield, Y, is not a true constant for heterogeneous populations. This differs from the concepts of Monod (20), Herbert, et al. (5), who have reported a constant value of Y for pure culture studies. Several investigators have noted the instability of the population in both continuous and batch operations with pure and

mixed cultures (13, 15, 16, and 89). The same authors have explained the changes in yield by diversion of some of the carbon source to time-dependent processes other than growth.

Marr, et al. (89) have considered the maintenance requirement as a possible explanation for the changing yield coefficient of Escherichia coli. They have proposed Equation 89 in which "specific maintenance rate" is taken into account. When the experimental data obtained in this research were plotted according to Equation 89, a straight line relationship was obtained up to a particular value of D. This graph is shown in Figure 65. It can be seen from the graph that a positive slope is obtained for the straight line $1/\bar{x}$ versus $\bar{t}$, indicating a decrease in yield coefficient at high detention times. The experimental data deviate from the straight line when the system is beginning to dilute out. Further, two different straight lines were obtained for the inflow substrate concentrations of 1000 and 3000 mg/l glucose. The values of specific maintenance rate for the two cases are 0.0154 and 0.0105 per hour, respectively. These values compare favorably with that of Marr, et al. (89) who reported a value of 0.0208 per hour for E. coli.

Hetling, et al. (15) have explained the change in yield coefficient as a result of change in basal metabolism, and death and lysis of cells; Figures 66 and 67 show

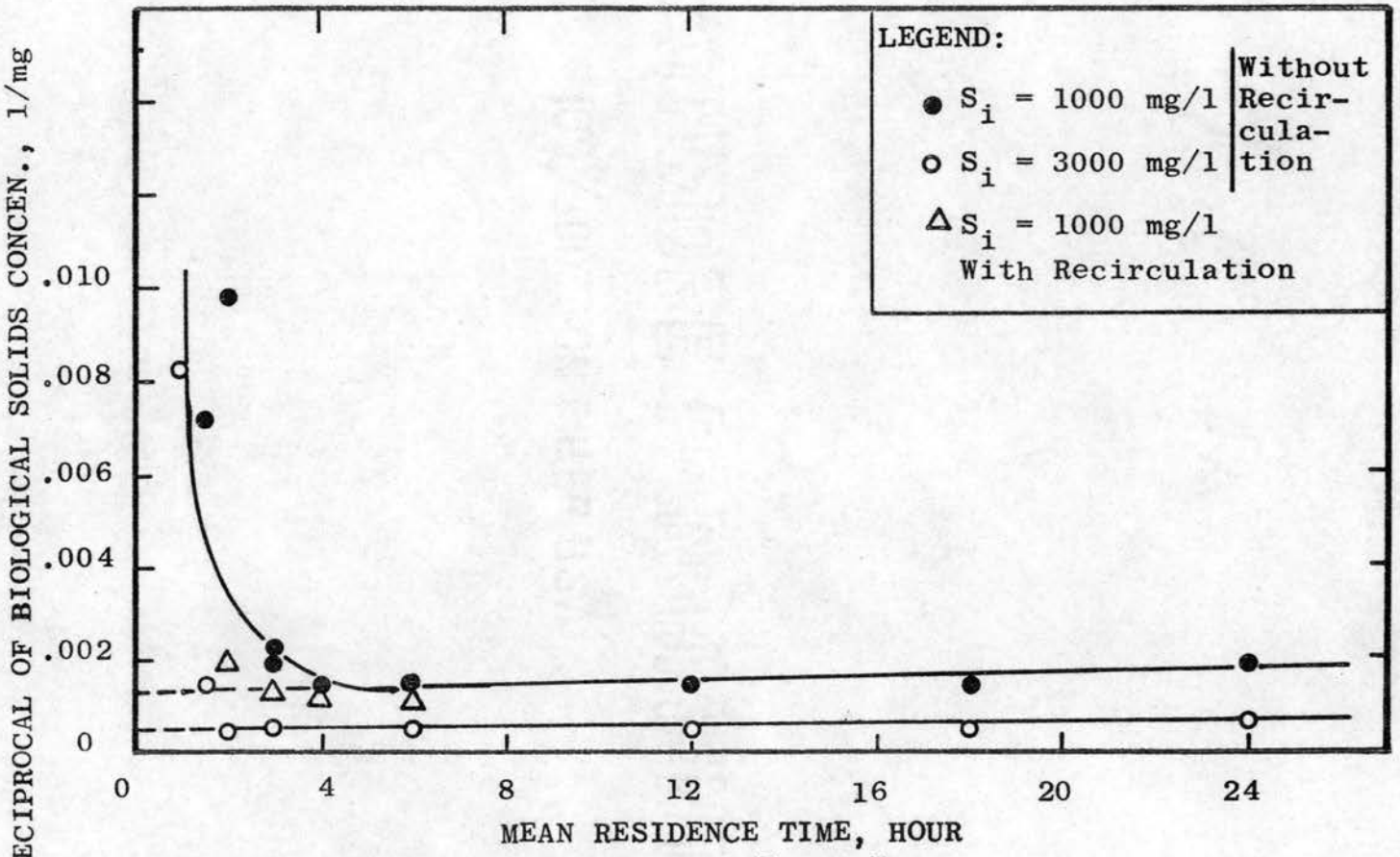

Figure 65. Relationship between $1/\bar{x}$ and $\bar{t}$.

plots of Equation 87, proposed by them. Figure 66 shows the reciprocal of the yield values obtained in both batch and continuous flow experiments plotted against mean residence time for the sludge developed using an inflow substrate concentration of 1000 mg/l glucose. Figure 67 shows values similarly obtained for the sludge developed with an inflow substrate concentration of 3000 mg/l glucose. It can be seen from these figures that the yield values obtained from batch experiments were lower than those from continuous flow experiments. Also, two separate straight lines were obtained for batch and continuous flow runs. There is an important difference between these graphs and Figure 65. In Figure 65 the experimental values of steady-state biological solids concentration deviates from a straight line at approximately the point where washout of cells begins; whereas in Figures 66 and 67 there is no such deviation, and the entire set of values is distributed along a straight line. This may indicate that yield coefficient provides a better measurement for determining maintenance requirement than solids concentration, since the solids level drops too rapidly in the washout region, whereas the yield coefficient does not. In the washout region, the drop in steady-state solids level is due primarily to the hydraulic flow rate through the reactor, while the yield coefficient apparently is not directly influenced by the hydraulic flow rate.

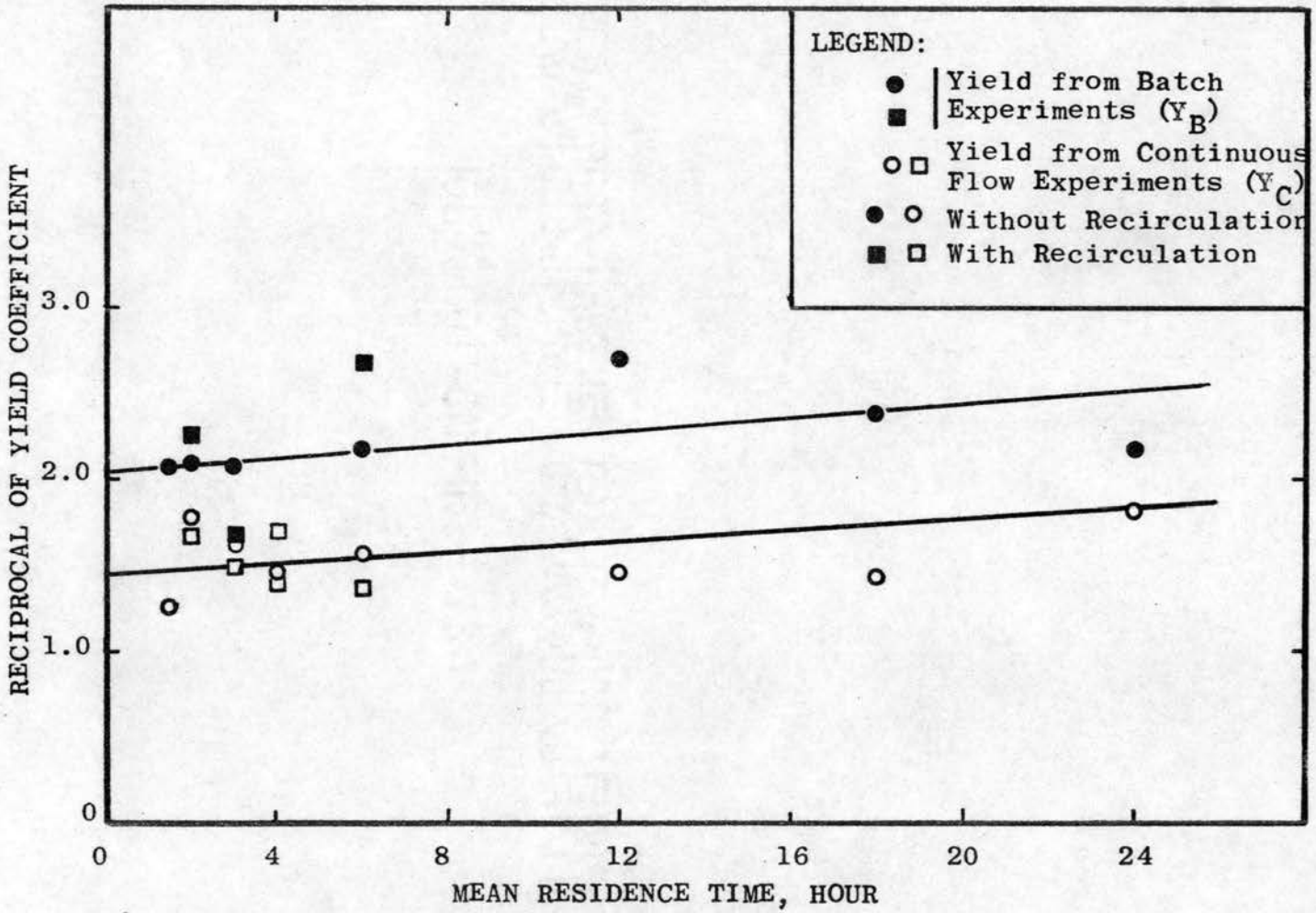

Figure 66. Relationship between 1/Y and Mean Residence Time ($\bar{t}$). (S_i = 1000 mg/l Glucose)

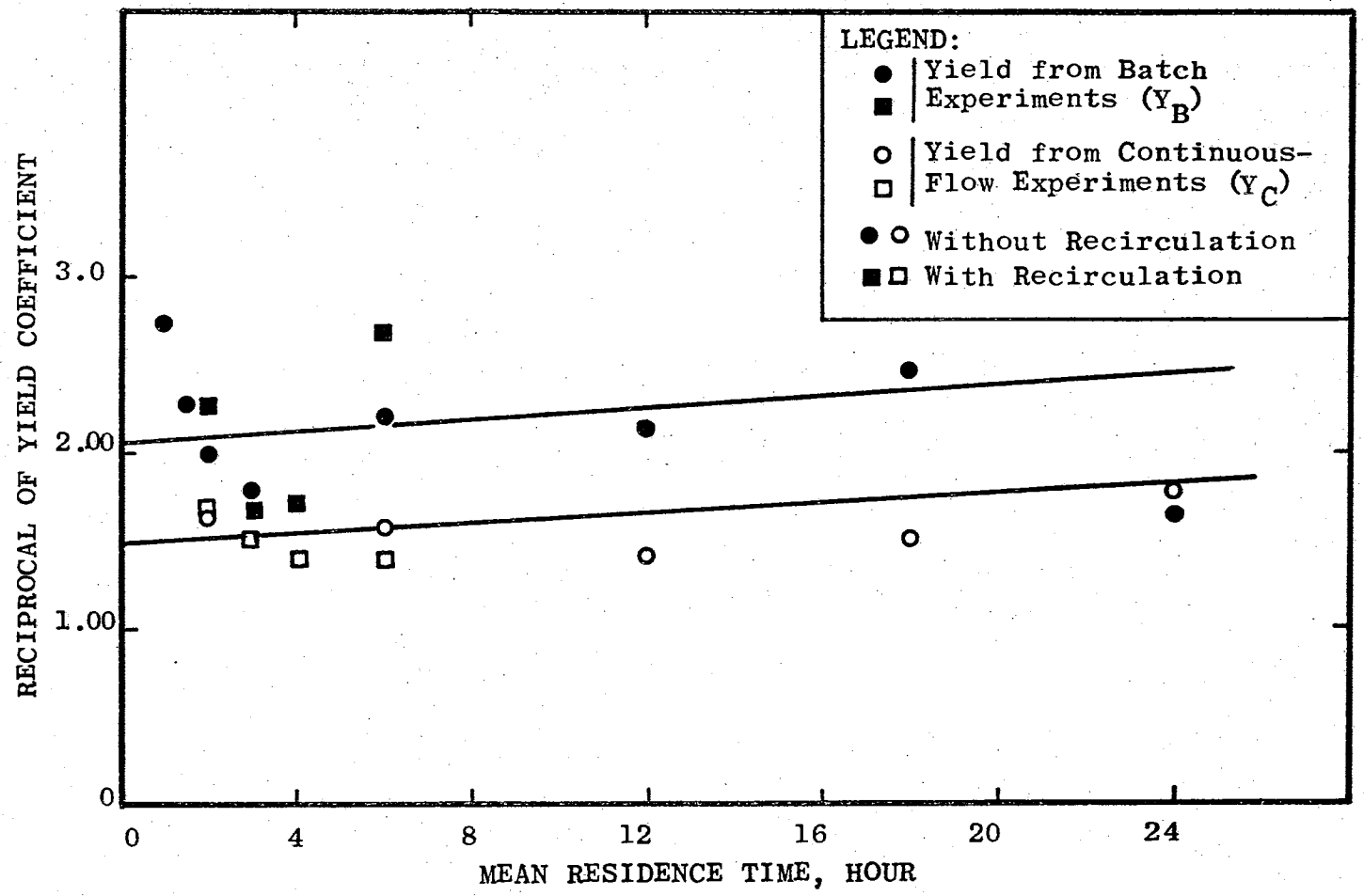

Figure 67. Relationship between 1/Y and Mean Residence Time. (S_i = 3000; without Recirculation)

The values of basal metabolic rate constants obtained from Y_B values are 0.02 and 0.019 per hour, respectively, for the cells harvested from steady-state units with inflow substrate concentrations of 1000 and 3000 mg/l glucose. The corresponding values determined from Y_C values are 0.017 and 0.014 per hour, respectively. The true yield coefficients (Y_t) calculated from the graphs are the same (0.488) for the batch runs conducted with the sludge developed with inflow substrate concentrations of 1000 and 3000 mg/l glucose. The corresponding values of Y_t for continuous flow experiments are 0.69 and 0.67, respectively. It appears from these figures that the equations proposed by Hetling, et al. (15) are more appropriate for describing the variations in yield coeffiencent than that of Marr, et al. (89).

4. Steady State Parameters at Different Dilution Rates

(a) Studies without Recirculation (S_i = 1000 mg/l Glucose)

Figure 68 shows the variations in the concentrations of biological solids, COD, carbohydrate, temperature, and pH during operation of a completely-mixed activated sludge unit at a dilution rate of 1/24 per hour. It can be seen from the figure that the values of COD and carbohydrate in the mixed liquor did not exhibit a large degree of fluctuation; however, the fluctuations in the concentration of biological solids are more distinct. It can also be seen

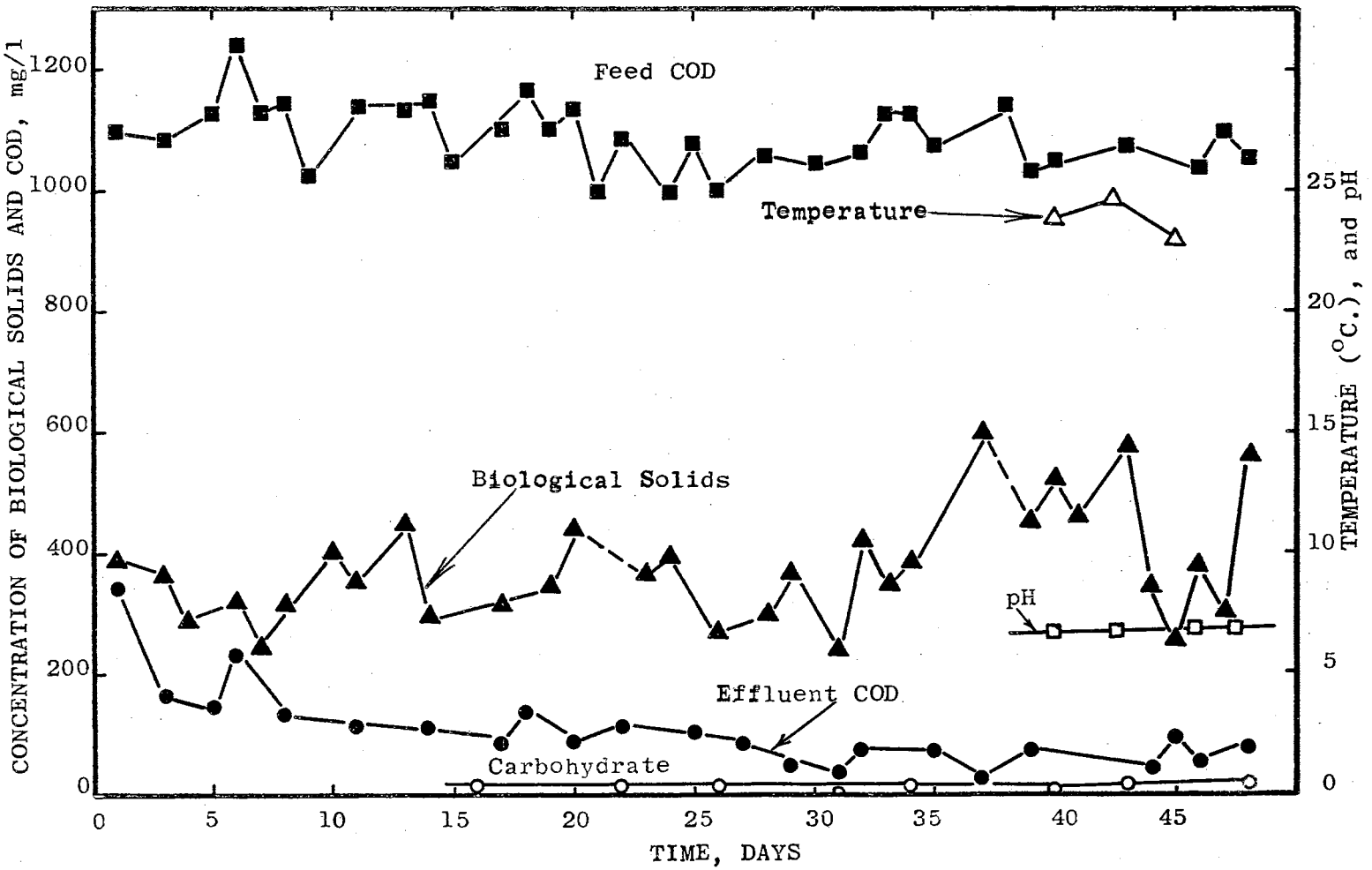

Figure 68. "Steady-State" Parameters at D = 1/24 Hour^{-1} without Recirculation. (S_i = 1000 mg/l Glucose)

that certain points are connected by broken lines in the solids curve. The broken lines are shown to represent a discontinuity in the system, due either to highly flocculated cells in the unit, or due to a failure in the system such as pumping or feed lines being clogged. It is proper to indicate here that the operation of a completely-mixed continuous flow process requires constant attention and supervision. Figure 68 shows that this particular study was conducted for 48 days, and some of the changes in the concentration of biological solids could be attributed to changes in predominant population and yield coefficients. It was intended to conduct long-term experiments so that a fairly accurate estimation of the steady-state parameters could be made. Statistical estimation of the mean values, standard deviation and coefficient of variance were made, and they will be presented later. Figure 69 shows the variation in steady-state parameters for another unit which was run at $D = 1/24\ hr^{-1}$. This experiment was made to find the differences in the biological solids concentration in the outflow and in the mixed liquor. It can be seen from the figure that the biological solids concentration in the mixed liquor was always greater than that in the effluent, but the fluctuations in solids concentration followed similar patterns in both the effluent and mixed liquor. In later experiments, solids concentrations were measured in both the effluent liquor and mixed liquor, and the mean

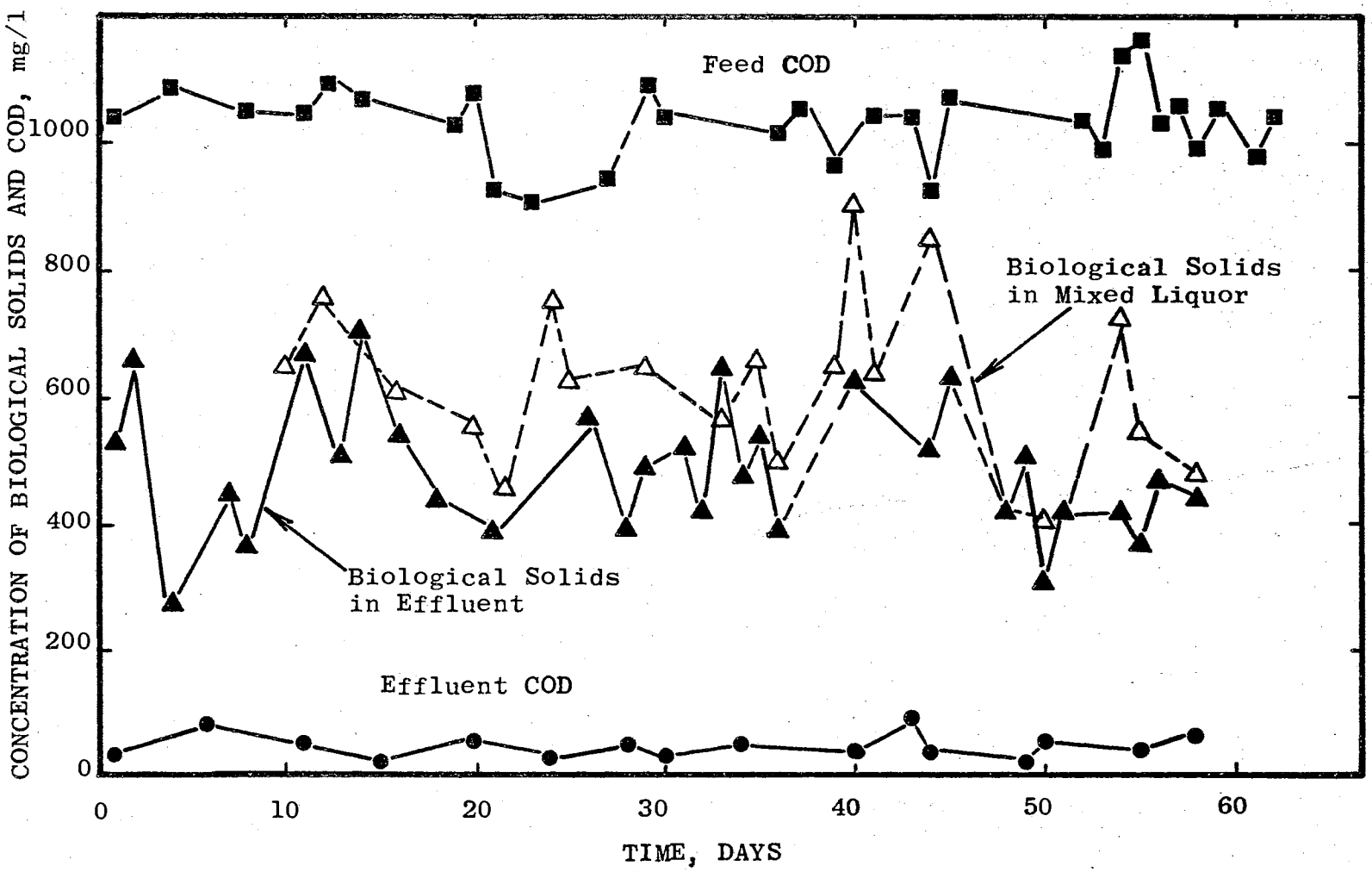

Figure 69. "Steady State" Parameters at D = 1/24 Hour^{-1} without Recirculation. (S_i = 1000 mg/l Glucose)

values were taken as the steady-state concentration of biological solids. Figure 69 also shows the variations in effluent COD and feed COD, and the fluctuations in these parameters are slight compared to those in biological solids concentration; variations in temperature, pH, and carbohydrate are shown in Figure 69A. It can be seen that the only parameter that is fluctuating considerably is the concentration of biological solids. Figures 70 through 77 show the results of similar studies at different dilution rates. At a dilution rate of 1.0 hr^{-1} the biological solids were washed out of the reactor, and the concentration of substrate became equal to that in the feed.

Table V shows the statistically estimated values of the steady-state parameters of the concentrations of substrate and biological solids. The concentrations of substrate are reported as both chemical oxygen demand ($\bar{S}$) and carbohydrate ($\bar{S}_c$). Also shown are the estimated mean values of substrate concentration in the feed and the coefficient of variance of each steady-state parameters. It can be seen from the table that the coefficients of variance for the carbohydrate concentration are high compared to those for solids and substrate concentration. This is due to the fact that the carbohydrate concentrations were generally very low and that even small differences in the absolute values are magnified in the calculations of variance. Also, it can be seen that the carbohydrate concen-

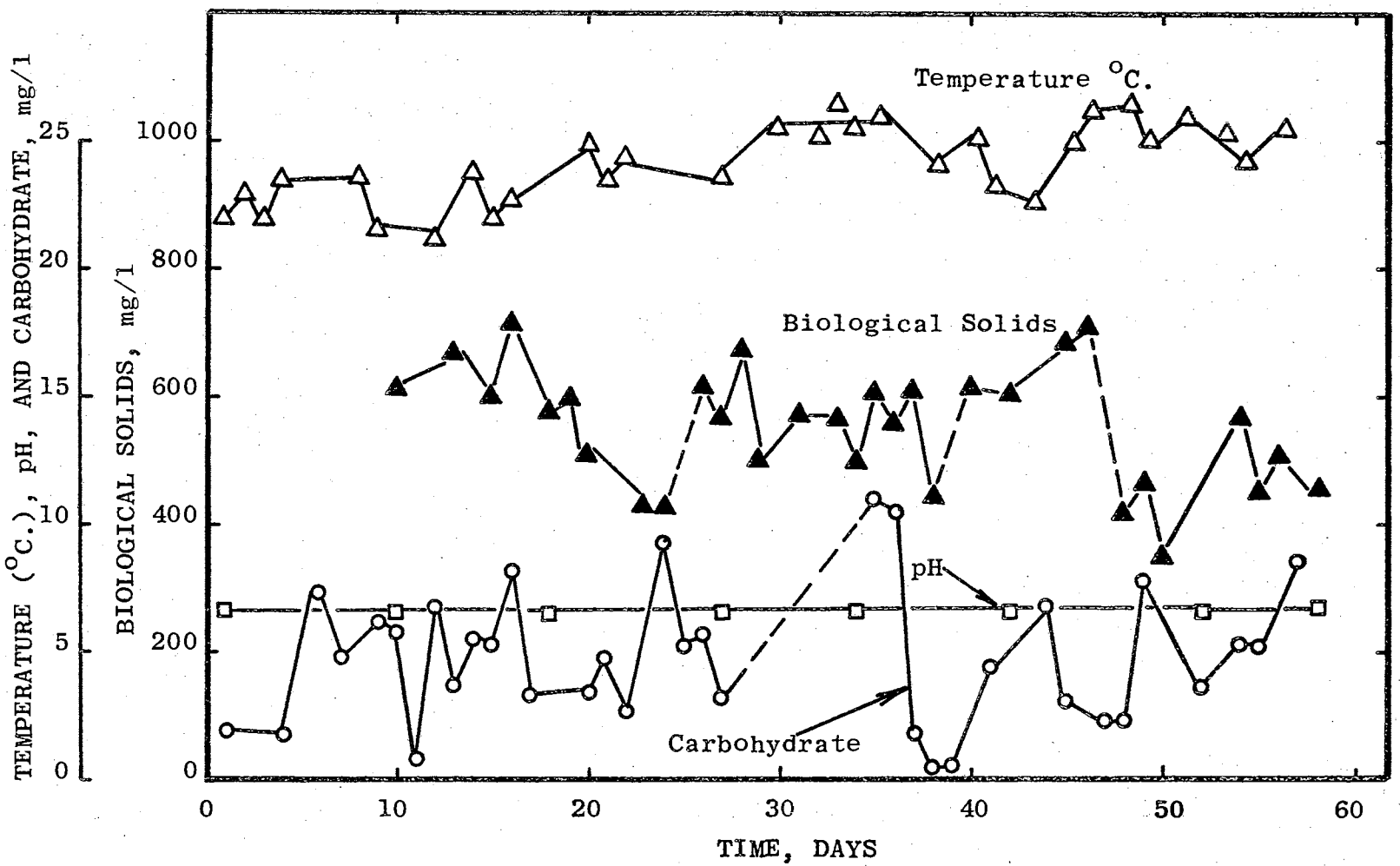

Figure 69A. "Steady State" Parameters at D = 1/24 Hour^{-1} without Recirculation. (S_i = 1000 mg/l Glucose)

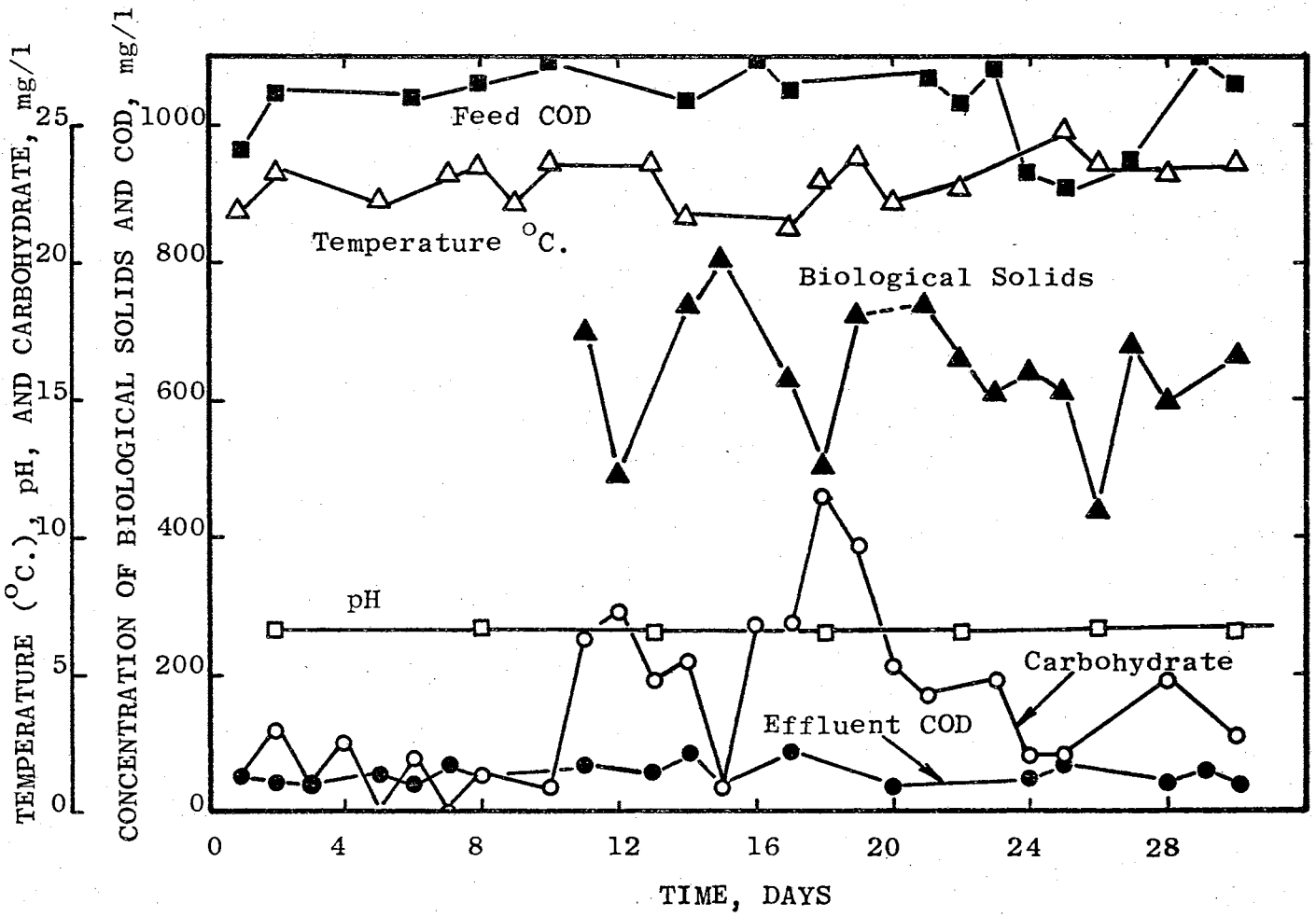

Figure 70. "Steady State" Parameters at D = 1/18 Hour^{-1} without Recirculation. (S_i = 1000 mg/l Glucose)

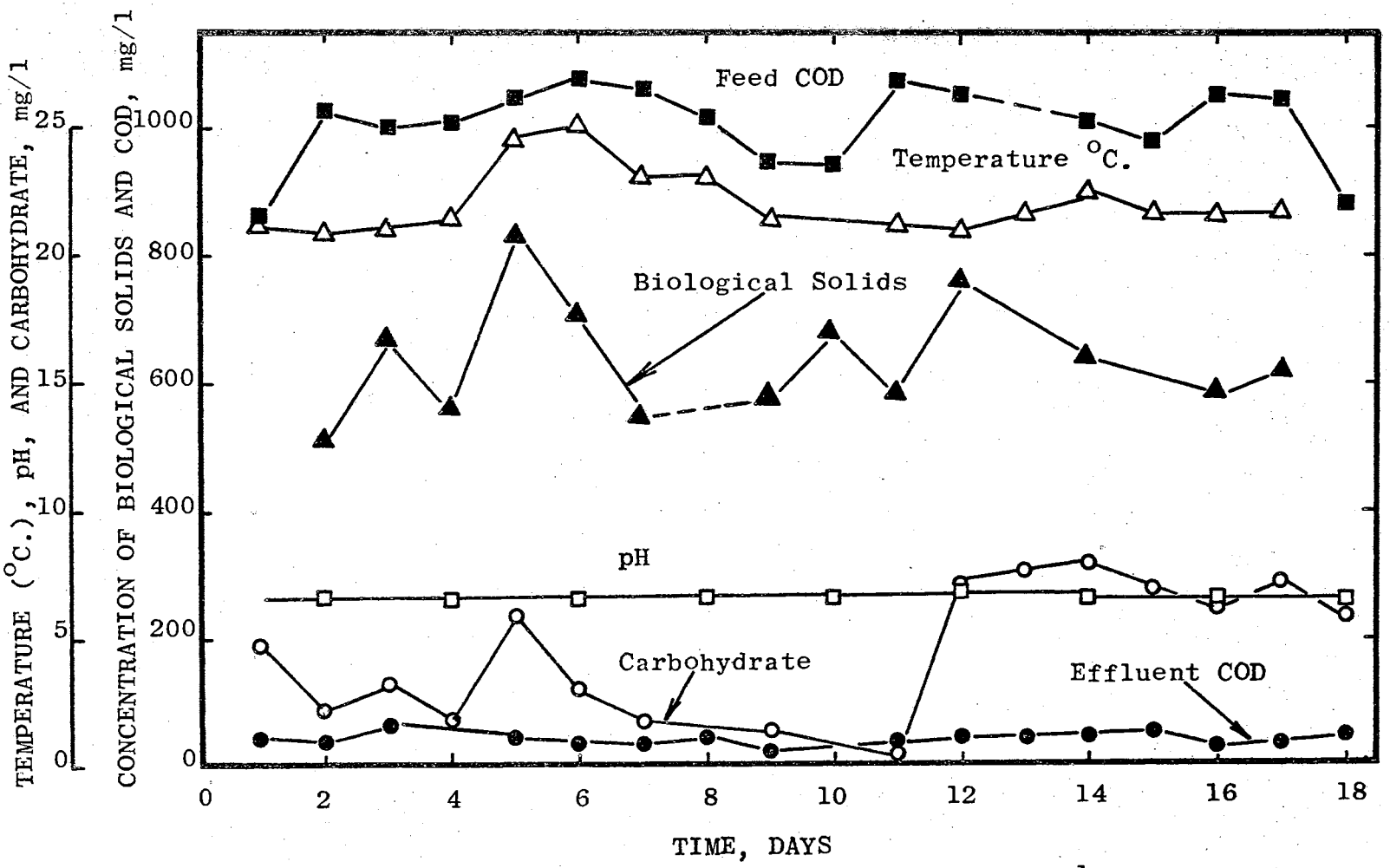

Figure 71. "Steady State" Parameters at D = 1/12 Hour^{-1}, without Recirculation. (S_i = 1000 mg/l Glucose)

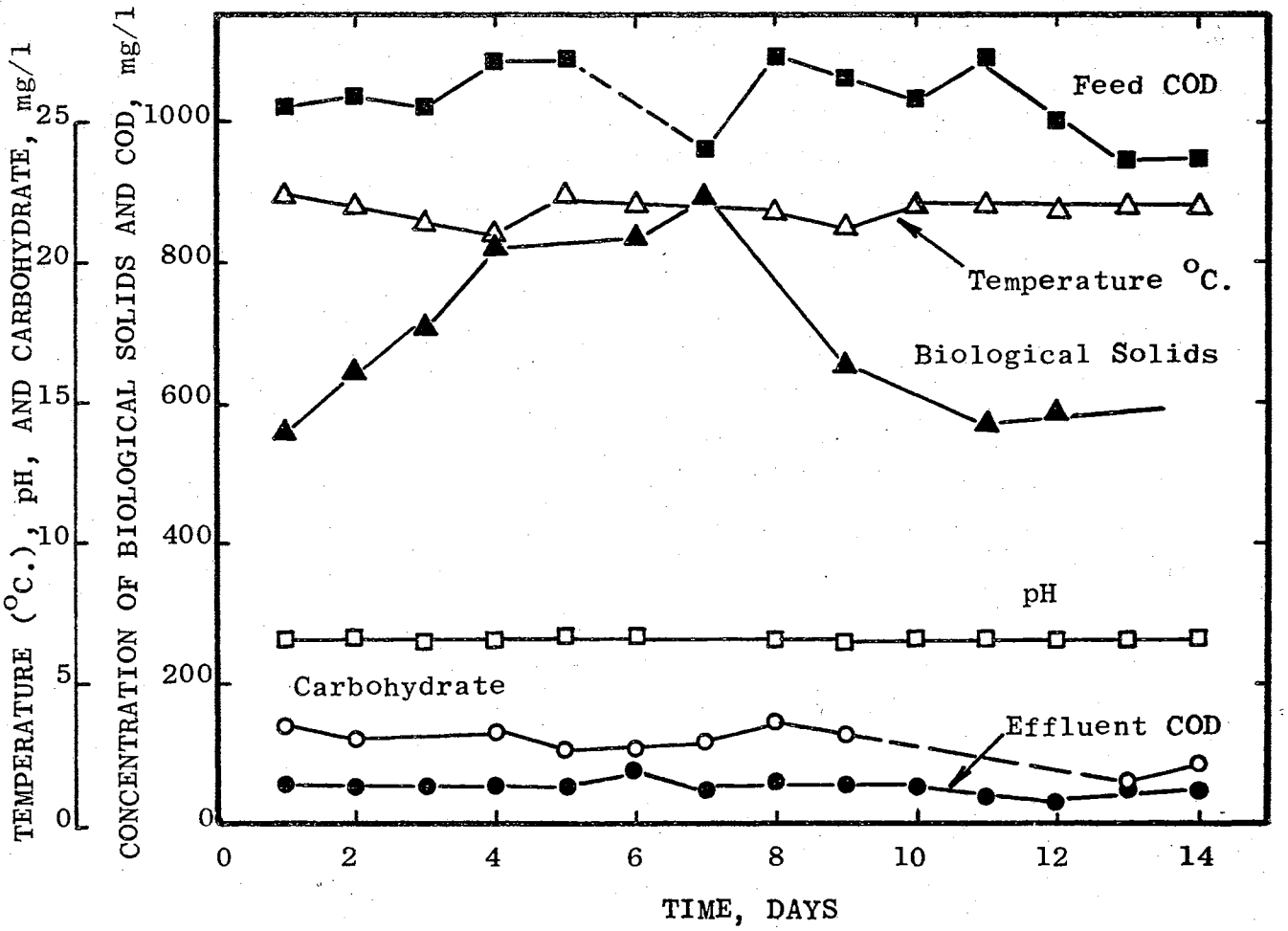

Figure 72. "Steady State" Parameters at D = 1/6 Hour^{-1} without Recirculation. (S_i = 1000 mg/l Glucose)

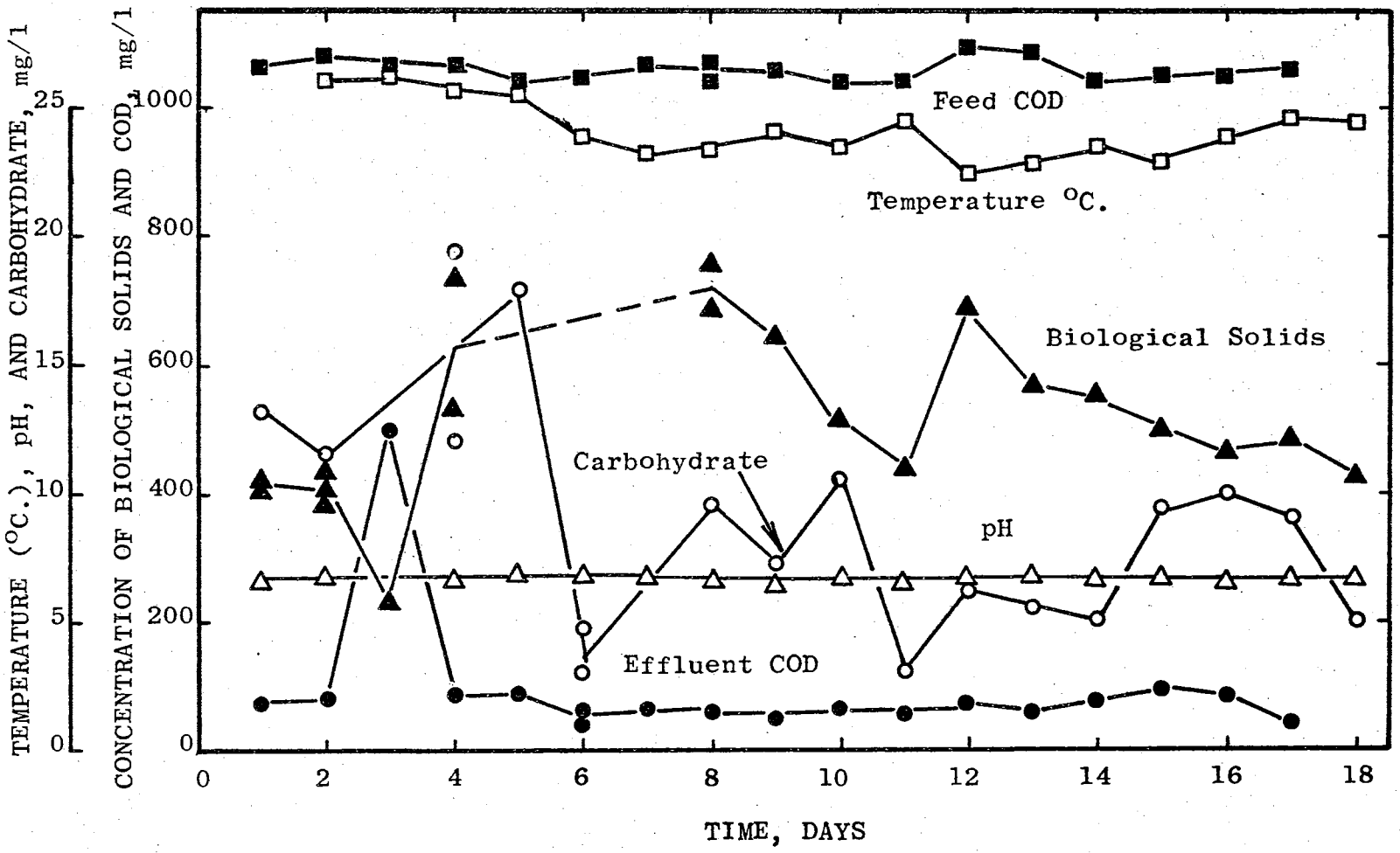

Figure 73. "Steady State" Parameters at D = 1/6 Hour^{-1} without Recirculation. (S_i = 1000 mg/l Glucose)

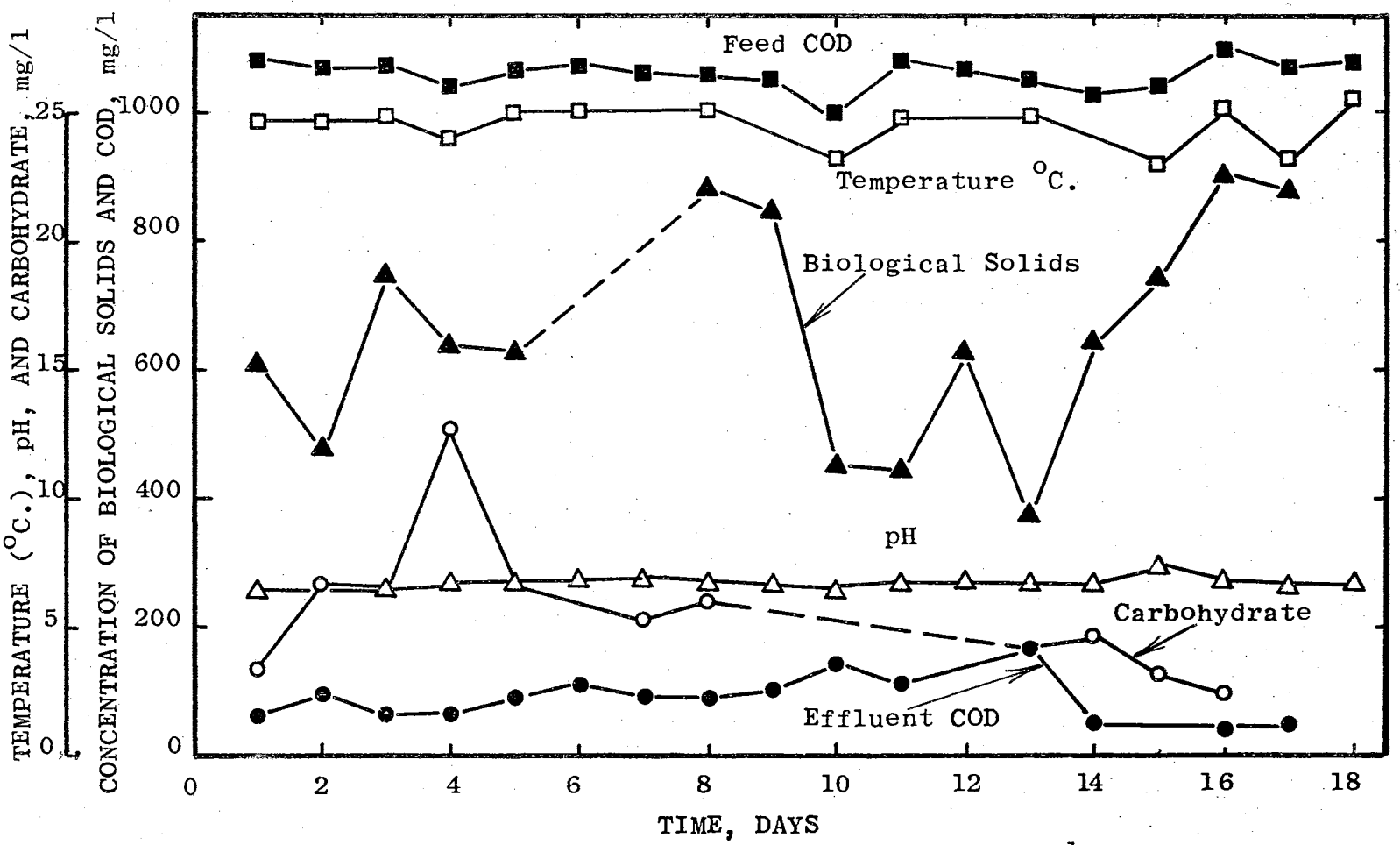

Figure 74. "Steady State" Parameters at D = 1/4 Hour^{-1} witout Recirculation. (S_i = 1000 mg/l Glucose)

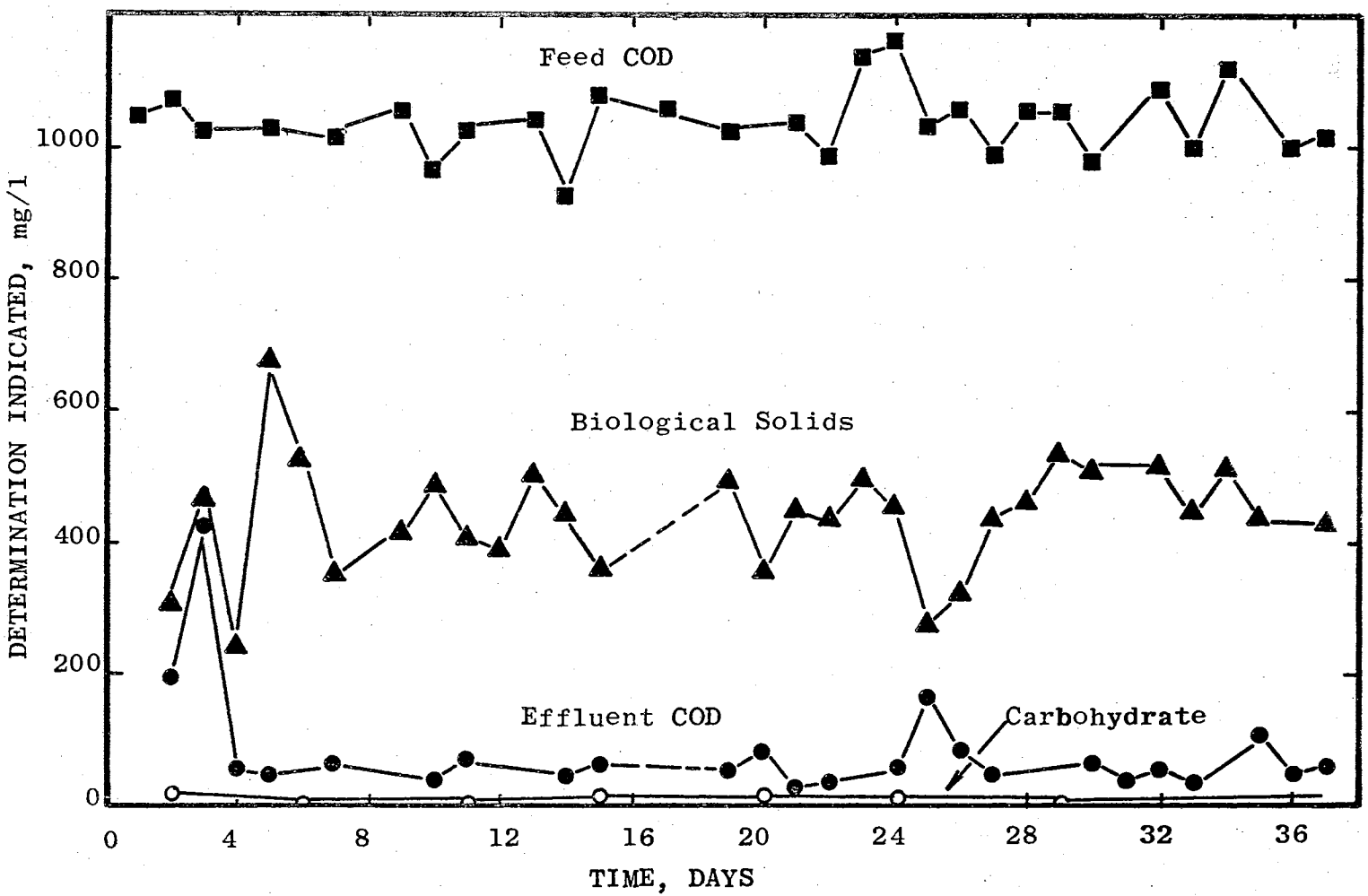

Figure 75. "Steady State" Parameters at D = 1/3 Hour^{-1} without Recirculation. (S_i = 1000 mg/l Glucose)

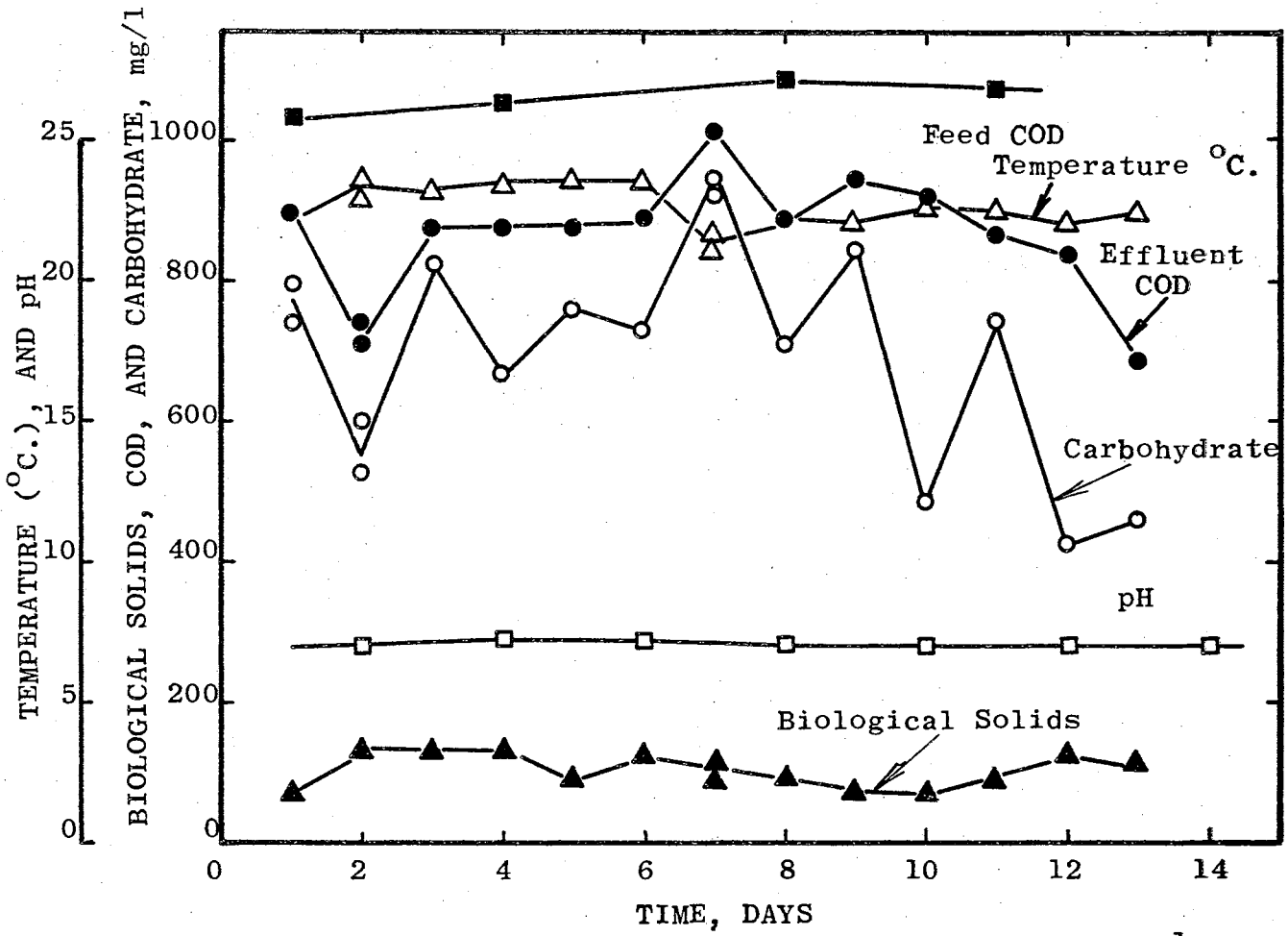

Figure 76. "Steady State" Parameters at D = 1/2 Hour^{-1} without Recirculation. (S_i = 1000 mg/l Glucose)

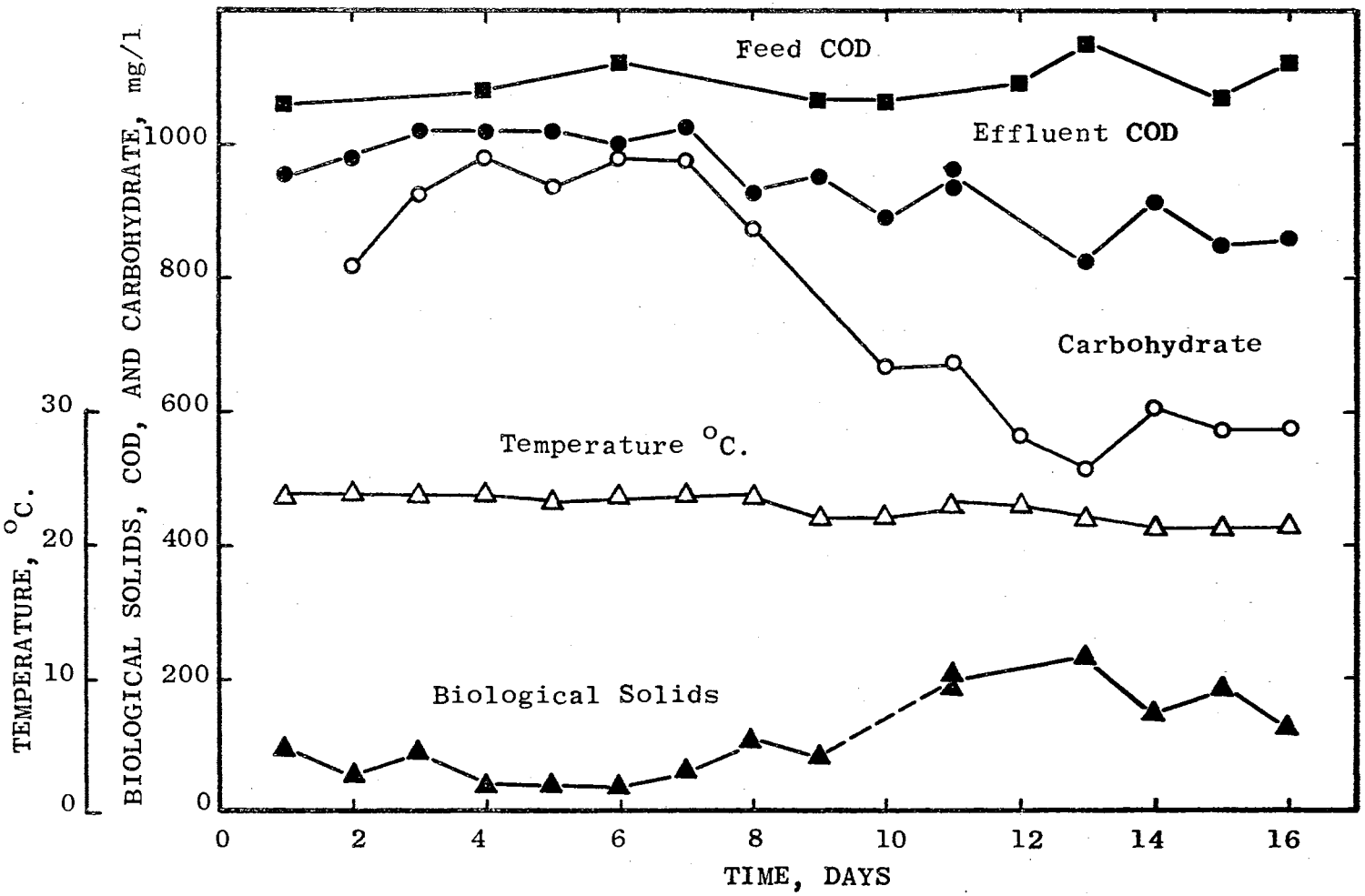

Figure 77. "Steady State" Parameters at D = 1/1.50 Hour^{-1} without Recirculation. (S_i = 1000 mg/l Glucose)

TABLE V

STATISTICAL ESTIMATION OF THE STEADY STATE PARAMETERS AT DIFFERENT DILUTION RATES

(S_i = 1000 mg/l Glucose; No Recirculation)

Dilution Rate Hr^{-1}		$\bar{x}$	$\bar{S}$	$\bar{S}_i$	$\bar{S}_c$	Coeffic. of Variance Per Cent			$\frac{D(S_i-\bar{S})}{\bar{x}}$	Loading Factor lbs COD/ lbs SS/day	COD Removal Efficiency %	Output Rate of Cells mg/l/hr
		mg/l	mg/l	mg/l	mg/l	x	S	S_c				
(1)		(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
1/24	0.042	540	48	1026	4.8	24.3	27.9	72.9	.076	4.33	95.5	22.65
1/18	0.056	687	54	1047	3.8	33.1	23.7	74.7	.0809	4.79	95.0	38.40
1/12	0.083	655	45	1015	4.3	16.6	21.7	64.4	.123	7.31	95.5	54.40
1/6	0.167	629	69	1058	9.20	46.7	21.9	45.70	.263	15.86	93.50	87.50
1/4	0.25	661	92	1057	8.40	26.2	38.5	92.8	.364	24.00	91.40	165.50
1/3	0.33	511	237	1067	213.8	39.5	86.2	103	.541	41.80	78.00	169.00
1/2	0.50	101	874	1057	698	24	11.9	22.9	.905	314	17.30	50.50
1/1.50	0.67	140	913	1091	764	71.3	11.50	24.1	.849	312	16.30	93.90

trations were generally very low and that even small differences in the absolute values are magnified in the calculation of variance. Also, it can be seen that the carbohydrate concentrations are always lower than COD values, indicating the presence of metabolic intermeidates at all dilution rates studied. When the concentrations of carbohydrate and COD began to increase during dilute-out of the culture, the coefficients of variance decreased, indicating that the low absolute values of the concentration are the reason for high values of variance at lower dilution rates. In column 9 of Table V are shown the values of specific substrate utilization rate calculated from the steady-state parameters. It can be seen from the table that the specific substrate utilization rate increased with increasing values of dilution rate. It is also shown in Table V that COD loadings as high as 24 lbs/ per lb. of biological solids per day can be applied with an efficiency greater than 90 per cent with respect to COD removal.

(b) Studies without Recirculation (S_i = 3000 mg/l Glucose)

Figures 78 through 85 show the variations in steady-state parameters at different dilution rates for a unit operating at an inflow substrate concentration of 3000 mg/l glucose. It can be seen from these figures that the fluctuations in biological solids concentration are more prominent than the fluctuations of effluent COD and carbohydrate concentration. Also, there was a corresponding

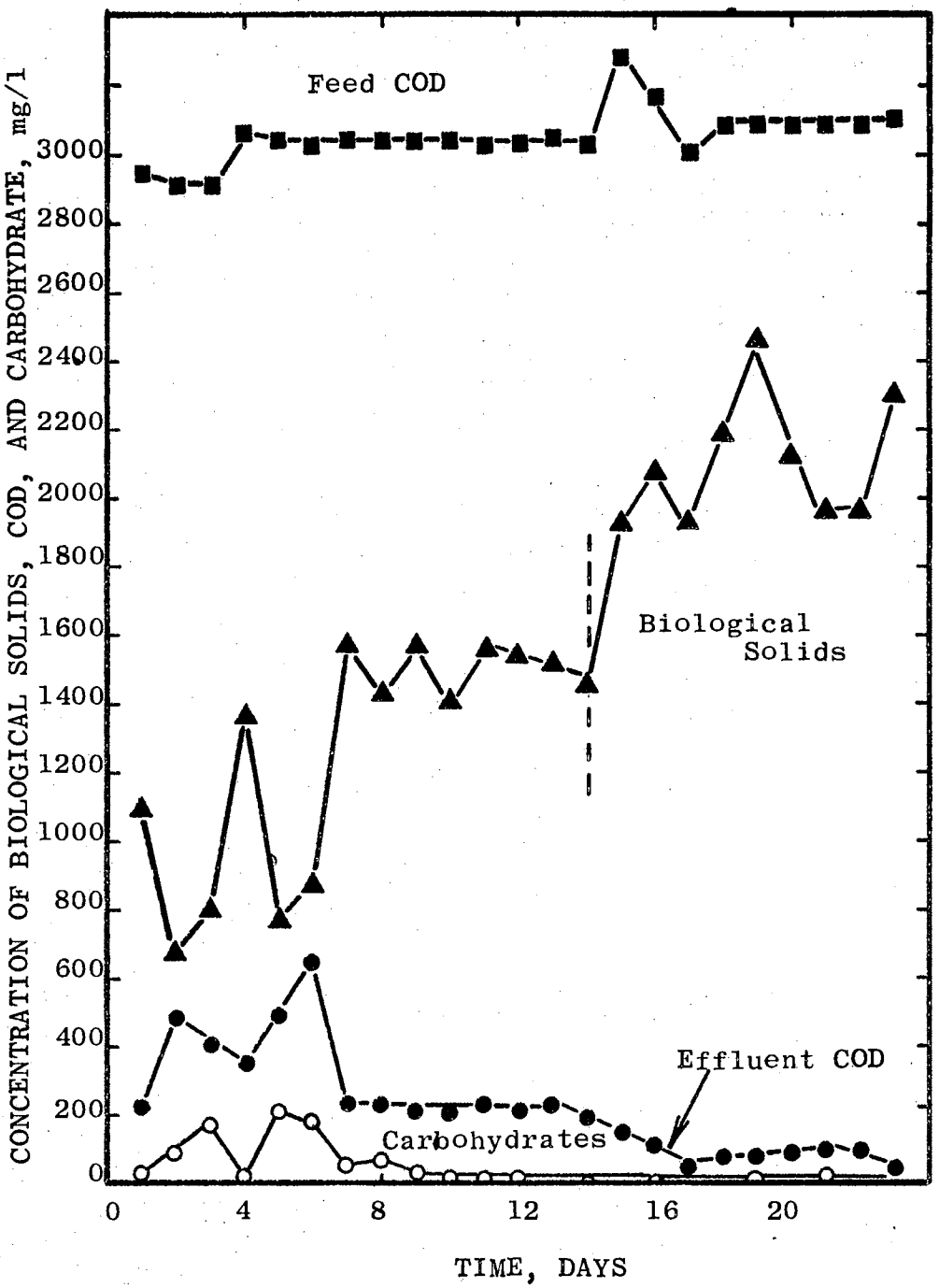

Figure 78. "Steady State" Parameters at $D = 1/24$ Hour^{-1} without Recirculation. (S_i = 3000 mg/l Glucose)

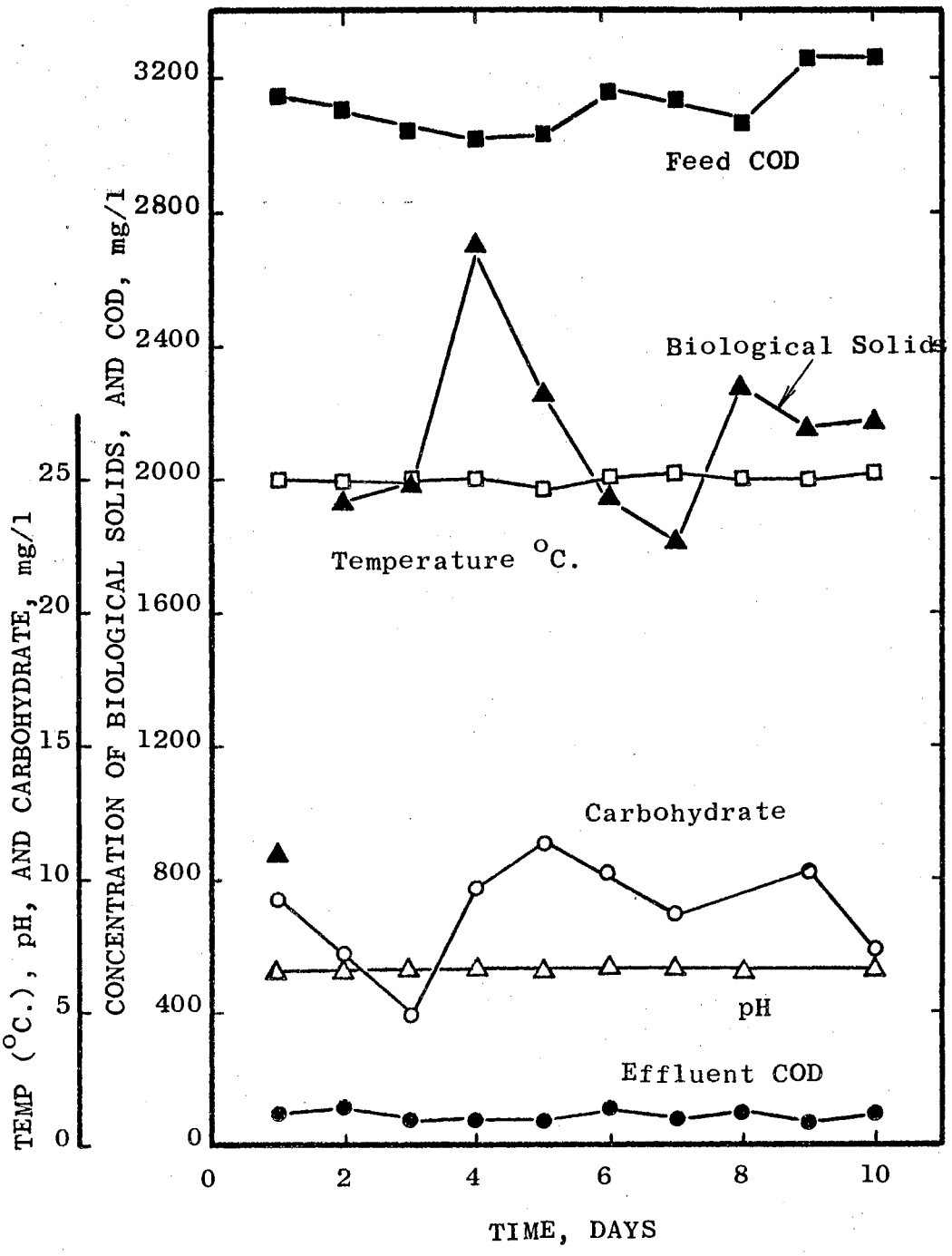

Figure 79. "Steady State" Parameters at $D = 1/18$ Hour^{-1} without Recirculation (S_i = 3000 mg/l Glucose)

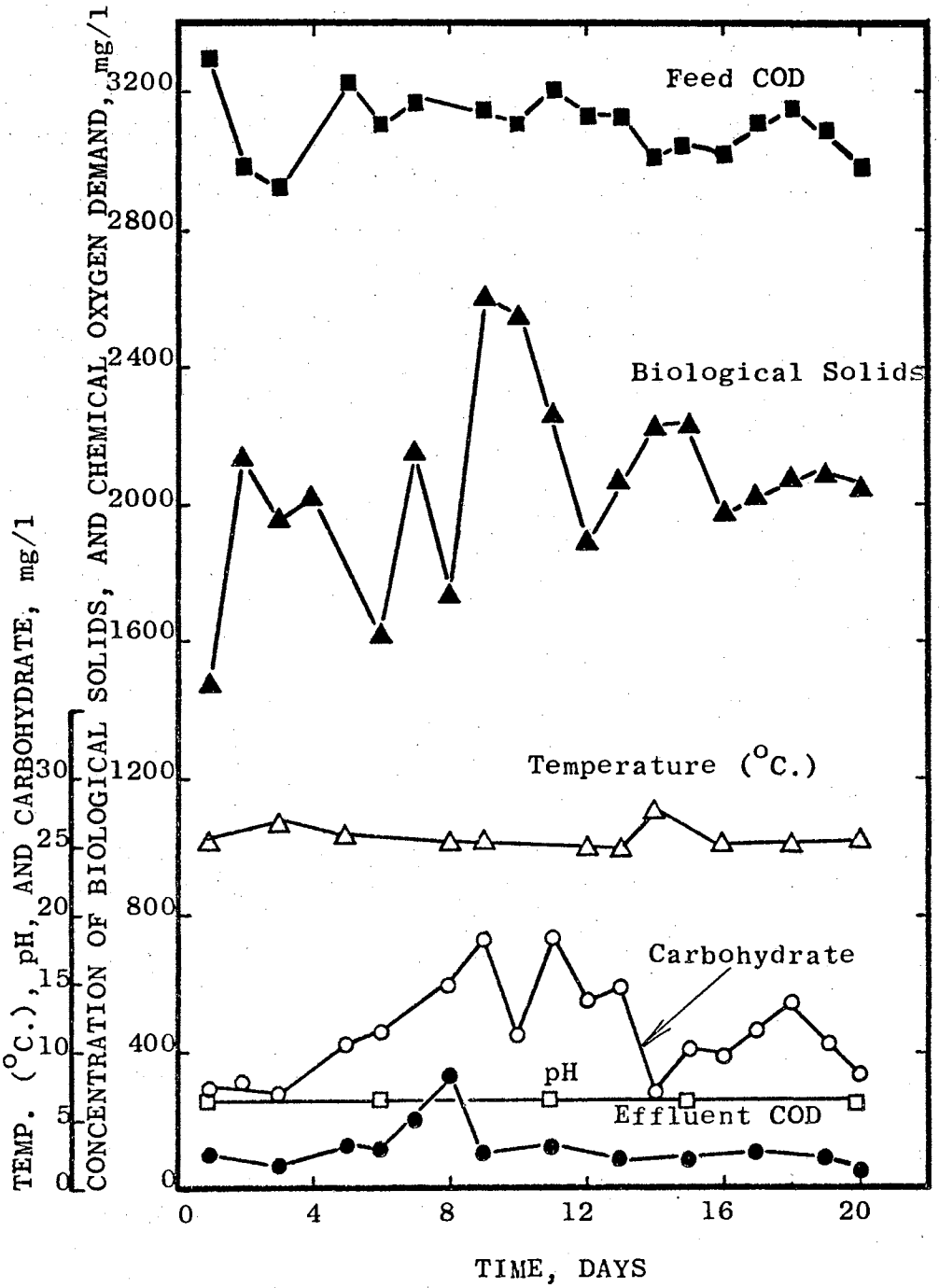

Figure 80. "Steady State" Parameters at D = 1/12 Hour-1 without Recirculation. (S_i = 3000 mg/l Glucose)

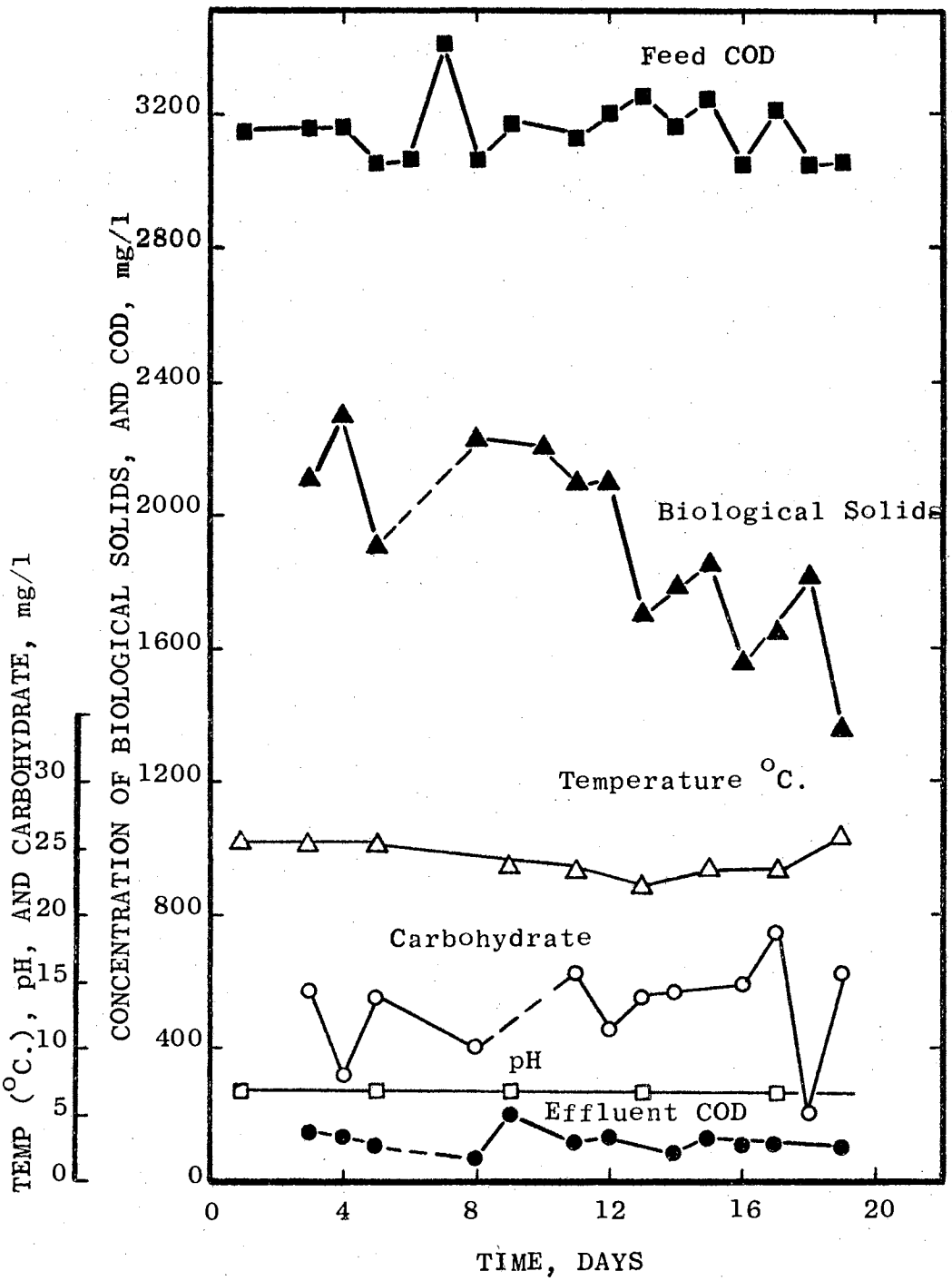

Figure 81. "Steady State" Parameters at $D = 1/6$ Hour^{-1} without Recirculation. (S_i = 3000 mg/l Glucose)

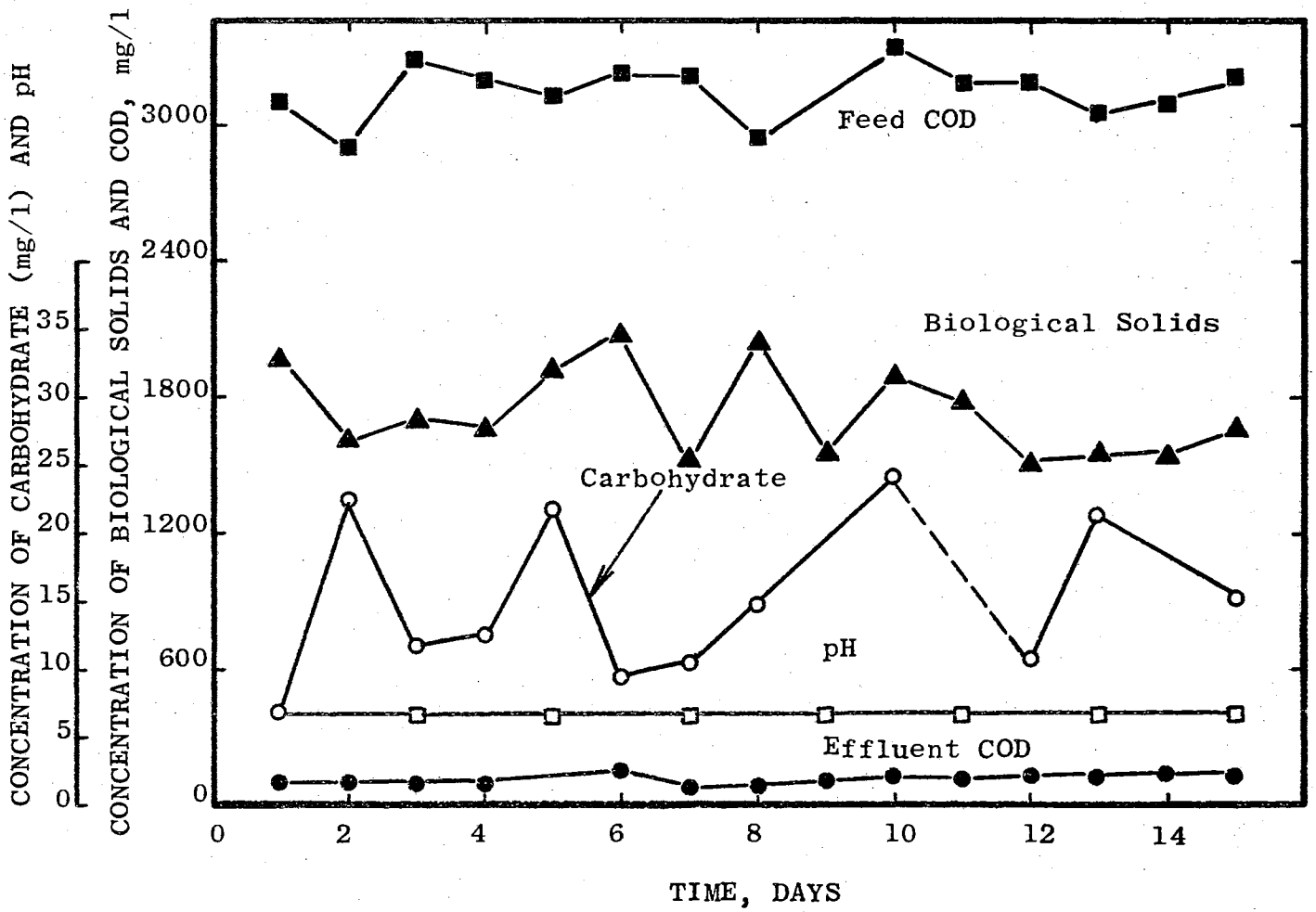

Figure 82. "Steady State" Parameters at D = 1/3 Hour^{-1} without Recirculation. (S_i = 3000 mg/l Glucose)

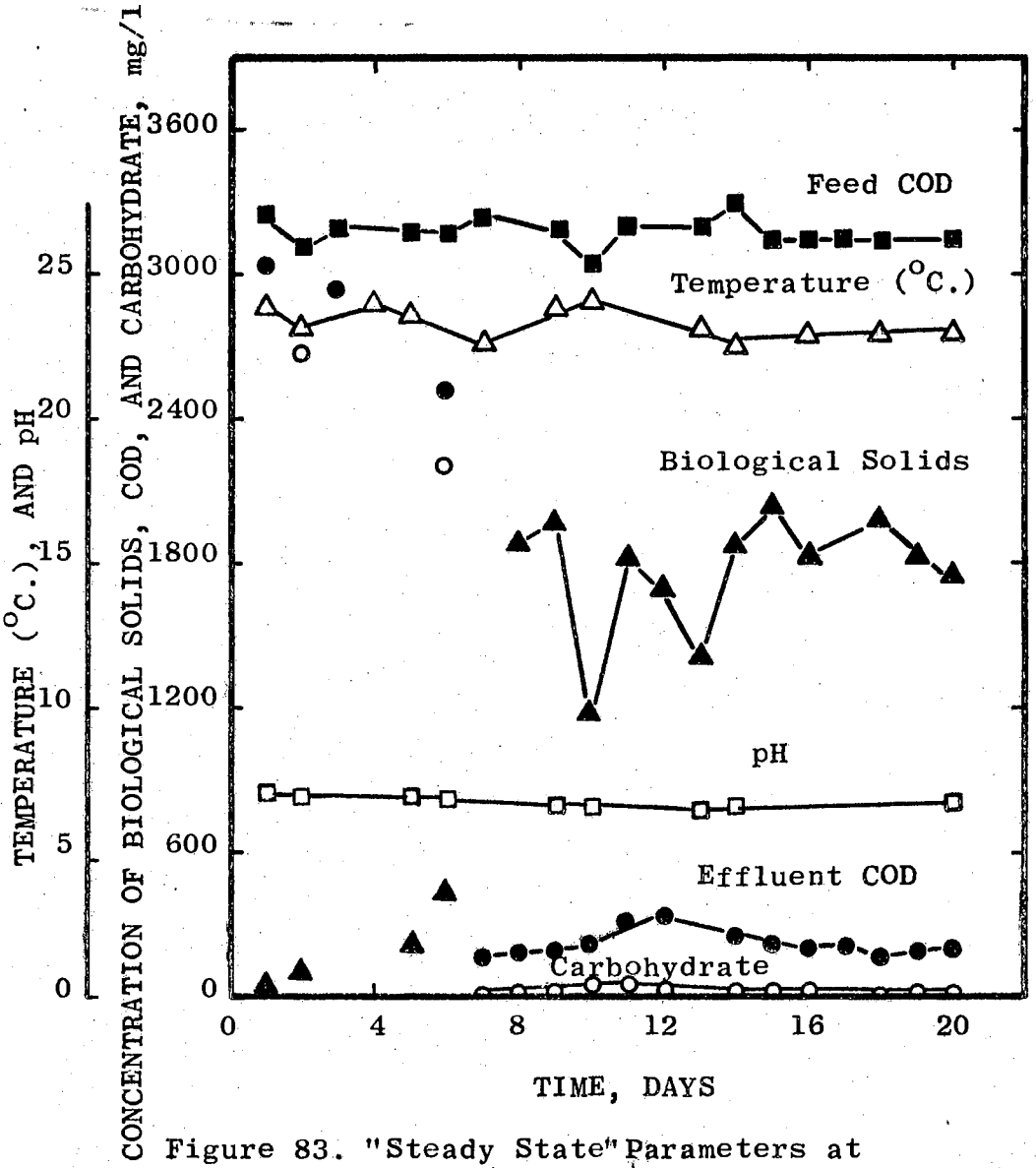

Figure 83. "Steady State" Parameters at $D = 1/2$ Hour^{-1} without Recirculation. (S_i = 3000 mg/l Glucose)

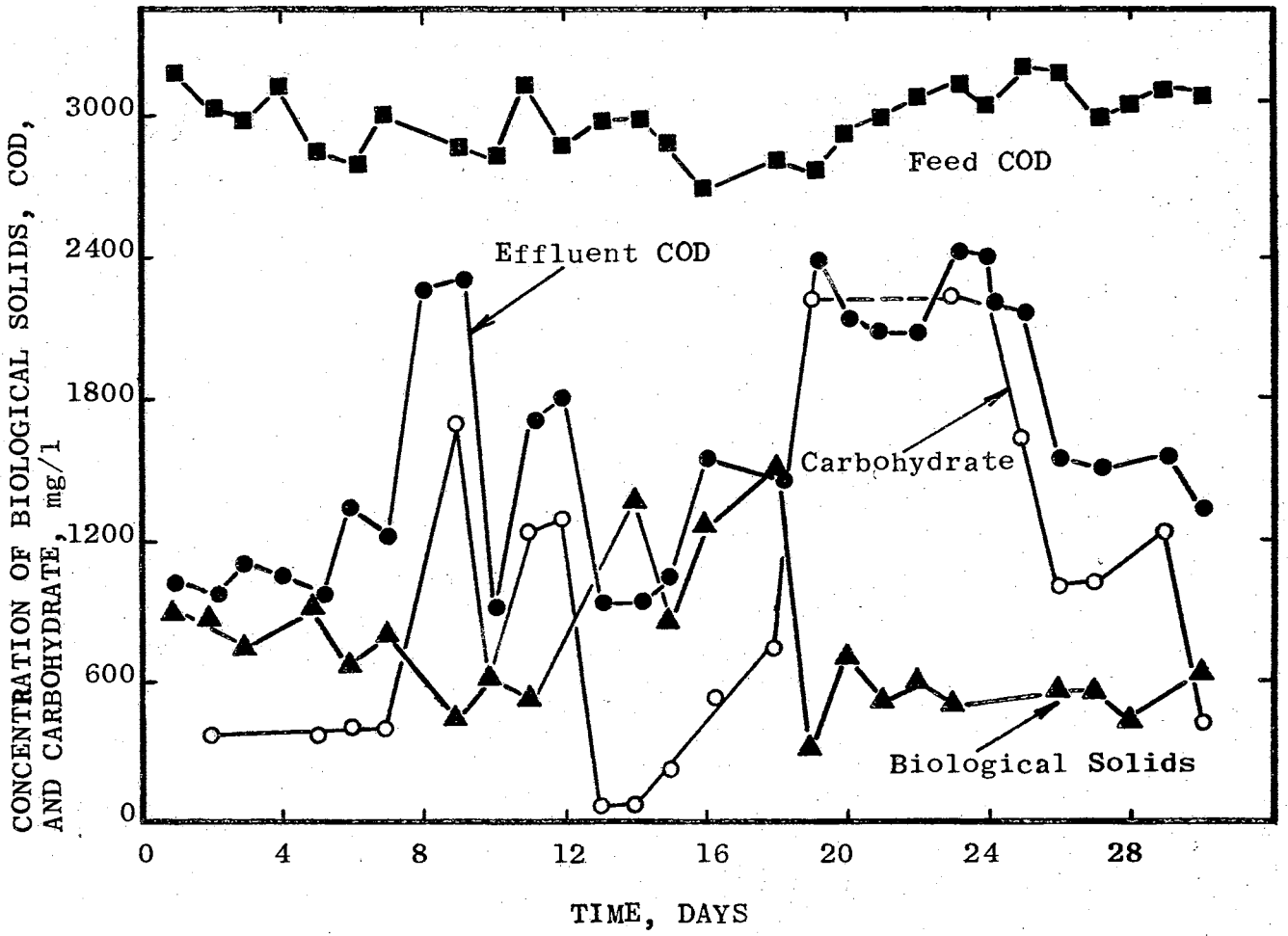

Figure 84. "Steady State" Parameters at D = 1/1.50 Hour^{-1} without Recirculation. (S_i = 3000 mg/l Glucose)

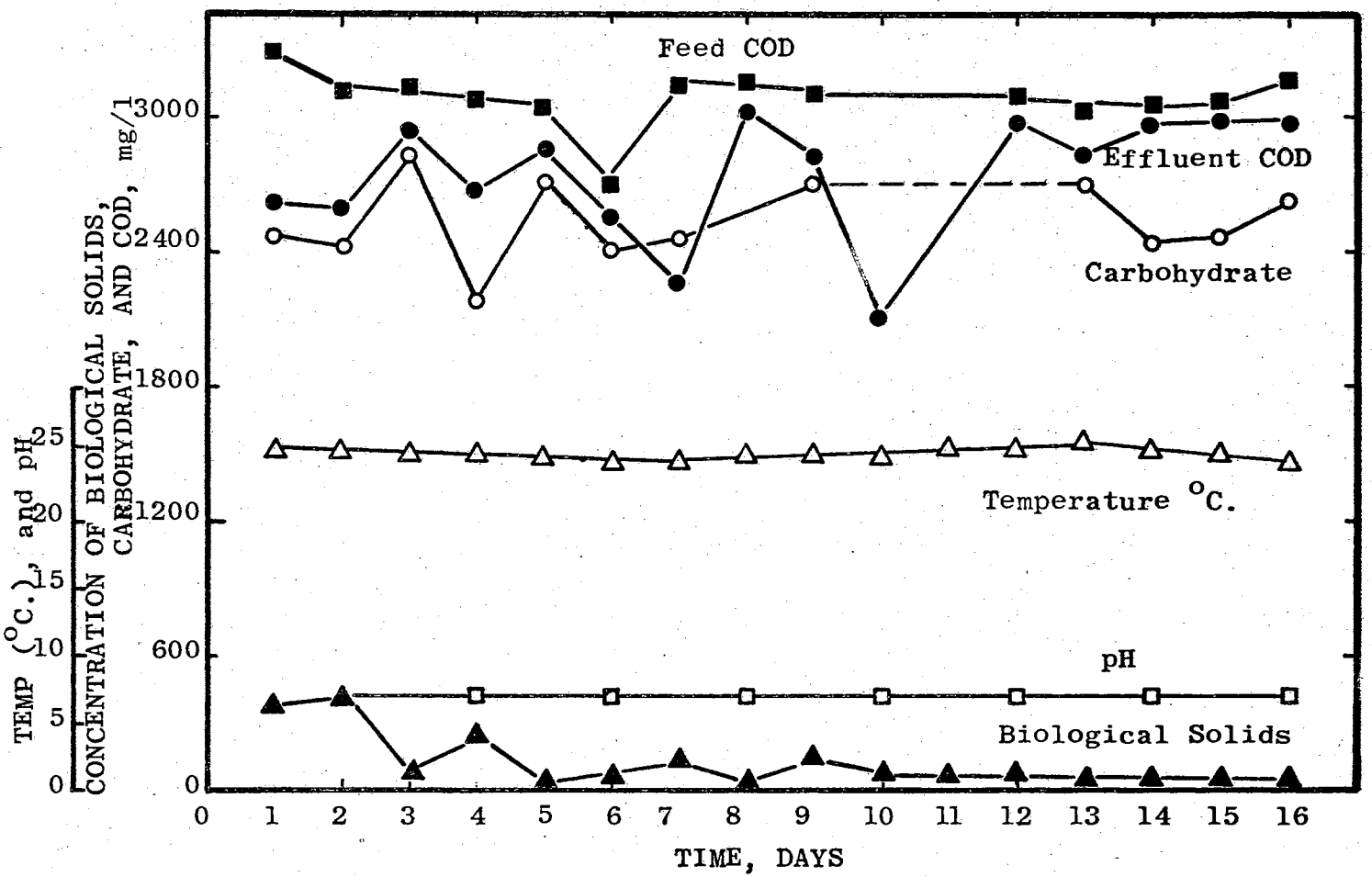

Figure 85. "Steady State" Parameters at D = 1.0 Hour^{-1} without Recirculation. (S_i = 3000 mg/l Glucose)

increase in numerical values of the system parameters due to the increase in the inflow substrate concentration; however, the increase was not always proportionate. A closer examination of Figure 78 shows that the concentration of biological solids increased during the first few days of operation and remained relatively steady until the 13th day. After this period the biological solids level increased to a higher value. This was due to the fact that the sludge in the reactor was highly flocculated, resulting in the increased solids level. The data collected up to 13 days of operation only were considered for the estimation of "steady-state" parameters. A summary of the average values of the steady-state parameters, and their coefficients of variance is shown in Table VI. The specific substrate consumption rate increases with increasing dilution rate as in the case of the system employing 1000 mg/l glucose as feed. Also, it is seen that COD loadings up to 53 lbs. per day per lb. of biological solids can be applied with an efficiency of COD removal greater than 92 per cent.

(c) Studies Employing Recirculation of Sludge (S_i = 1000 mg/l Glucose)

Four experiments were conducted in which sludge recirculation was employed. These experiments were run at mean residence times of 6, 4, 3, and 2 hours. Figures 86 through 90 show the steady-state parameters at different dilution rates. In addition to solids concentration in the

TABLE VI

STATISTICAL ESTIMATION OF THE STEADY-STATE PARAMETERS AT DIFFERENT DILUTION RATES

(S_i = 3000 mg/l Glucose; No Recirculation)

Dilution Rate Hr^{-1}		$\bar{x}$ mg/l	$\bar{S}$ mg/l	$\bar{S}_i$ mg/l	$\bar{S}_c$	Coefficient of Variance x	Coefficient of Variance S	Coefficient of Variance $\bar{S}_c$	Yield Y_c	$D(S_i-\bar{S})/\bar{x}$	Loading Factor lbs COD/ lbs SS/day	COD Removal Efficiency %	Rate of Output of Cells mg/l/hr
(1)		(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
1/24	0.042	1589	221	3048	43.40	32.2	71.3	144.0	0.562	.0726	4.54	92.60	66.6
1/18	0.056	2010	87	3121	8.60	23.4	18.4	23.2	0.664	.0845	4.89	97.0	112.50
1/12	0.083	1993	112	3086	11.20	19.7	54.5	32.8	0.670	.1240	7.31	96.50	165.50
1/6	0.167	1917	120	3147	17.4	20.2	28.4	69.5	0.633	.2640	15.50	96.20	320
1/3	0.333	1731	113	3139	17.3	11.7	20.3	45.4	0.572	.5820	34.20	96.40	576
1/2	0.500	1787	224	3163	31.0	13.6	23.7	38.9	0.608	.8230	53.20	92.90	893
1/1.50	0.667	676	1569	2950	1225.0	46.4	36.5	74.5	0.490	1.36	174.8	46.80	451
1	1.00	122	2745	3080	2535	97.1	10.1	7.1	0.365	2.74	1510	10.90	122

mixed liquor, the values of solids concentration in the return sludge are also plotted. In general, the solids concentration in the reactor increased as a result of recirculation, thereby reducing the loading factor on the system. It can also be seen from the graphs that the fluctuations in solids concentration are larger than for other parameters. The above studies were conducted with a recirculation ratio of 0.25 and a concentration factor of 1.50. At a mean residence time of 3 hours, it was not possible to attain a concentration factor of 1.50 during certain periods of the experiment; therefore, the experiment was conducted at c = 1.0 until such time as the solids in the return sludge could be increased. This operational problem was due to poor settleability of the sludge. The results of this experiment are shown in Figure 89. It can be seen from the figure that both the mixed liquor solids and recycle solids concentrations are the same. Figures 90A and 90B show the variations in system parameters with the reactor running at a mean residence time of 2 hours. There seems to be a cyclic variation in the concentration of solids as well as COD which is different from other experiments. Table VII shows the values of the specific substrate consumption rate, loading factor, and efficiency of COD removal for studies employing recirculation of sludge. It can be seen that the specific substrate consumption rate increases with increasing dilution rate and

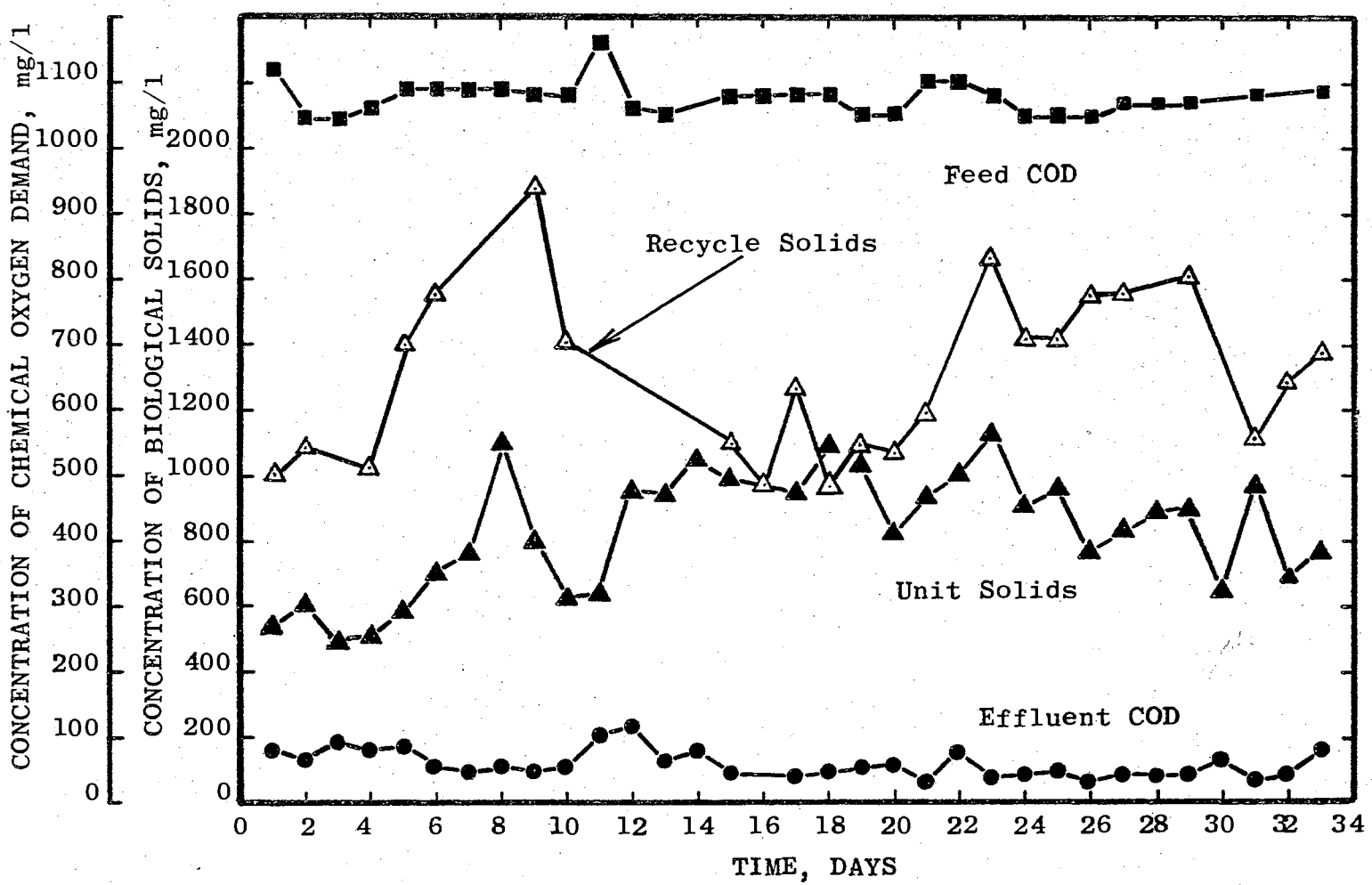

Figure 86. "Steady State" Parameters at $\bar{t}$ = 6 Hours with Recirculation. (S_i = 1000 mg/l Glucose; a = 0.25; c = 1.50)

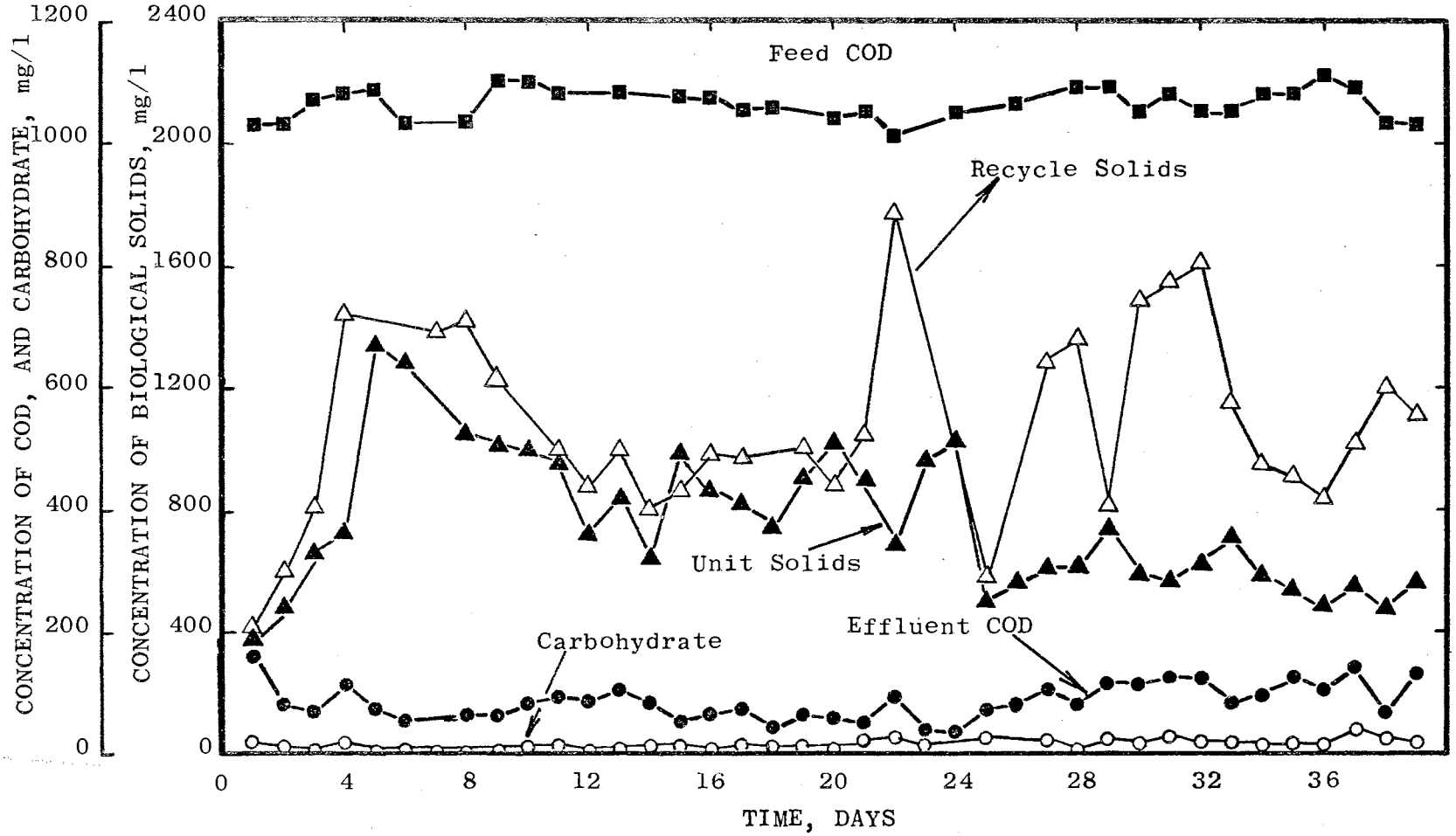

Figure 87. "Steady State" Parameters at $\bar{t}$ = 4 Hours with Recirculation. (S_i = 1000 mg/l Glucose; α = 0.25; c = 1.50)

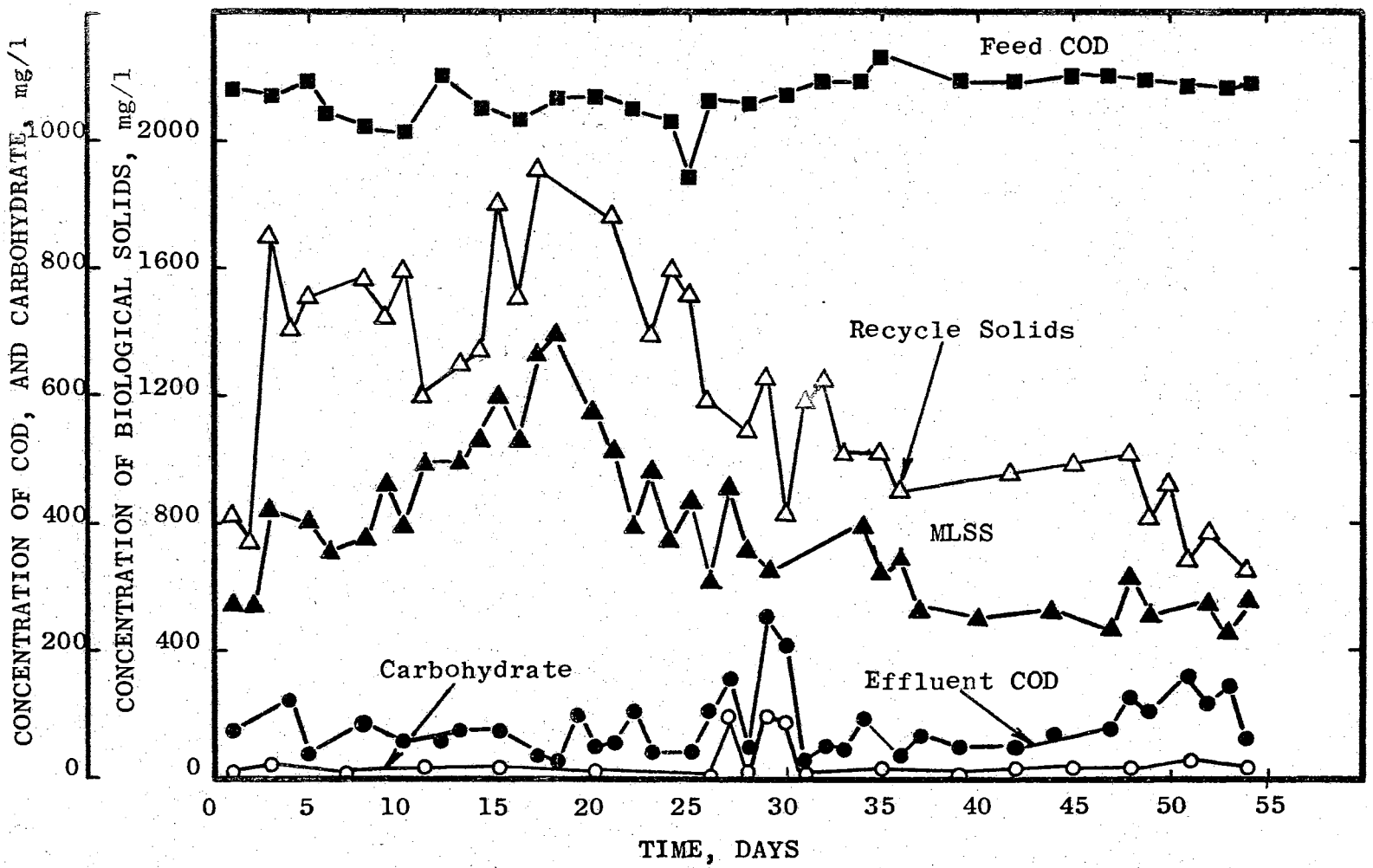

Figure 88. "Steady State" Parameters at $\bar{t}$ = 3 Hours with Recirculation. (S_i = 1000 mg/l Glucose; α = 0.25; c = 1.50)

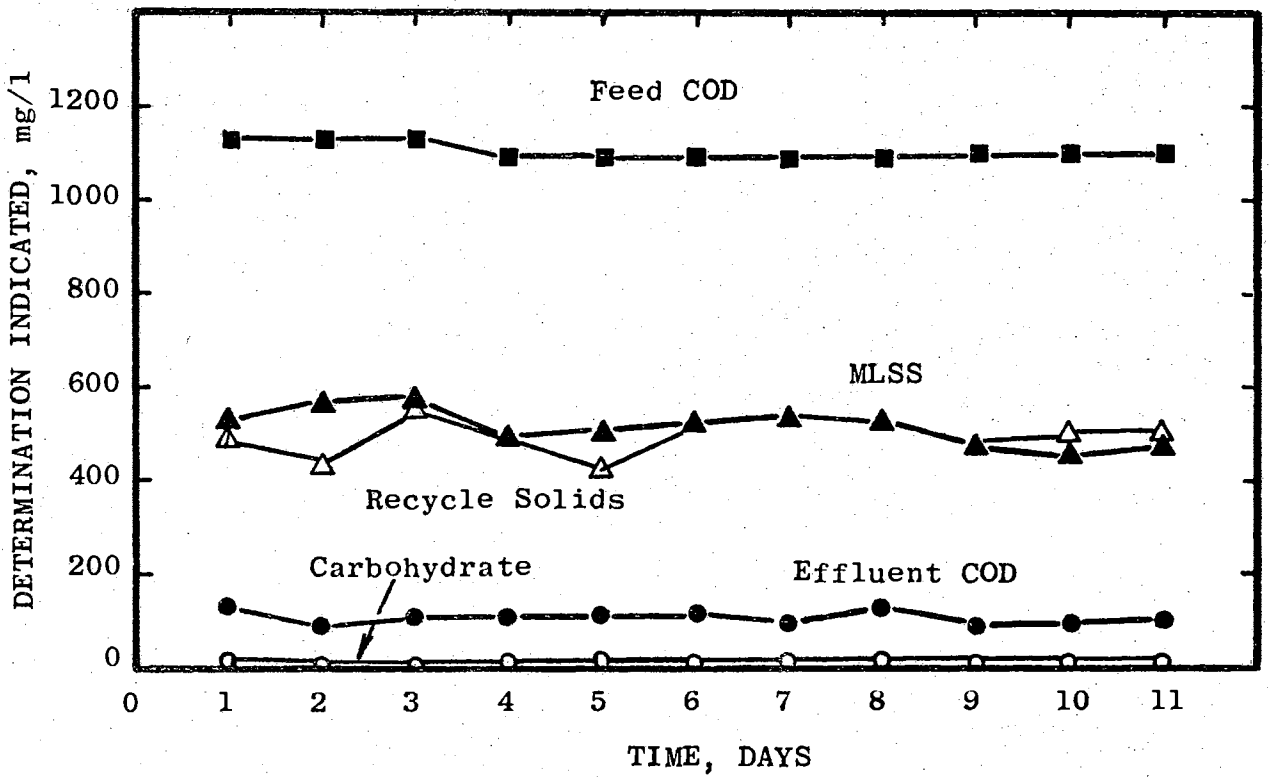

Figure 89. "Steady State" Parameters at $\bar{t}$ = 3 Hours with Recirculation. (S_i = 1000 mg/l; α = 0.25)

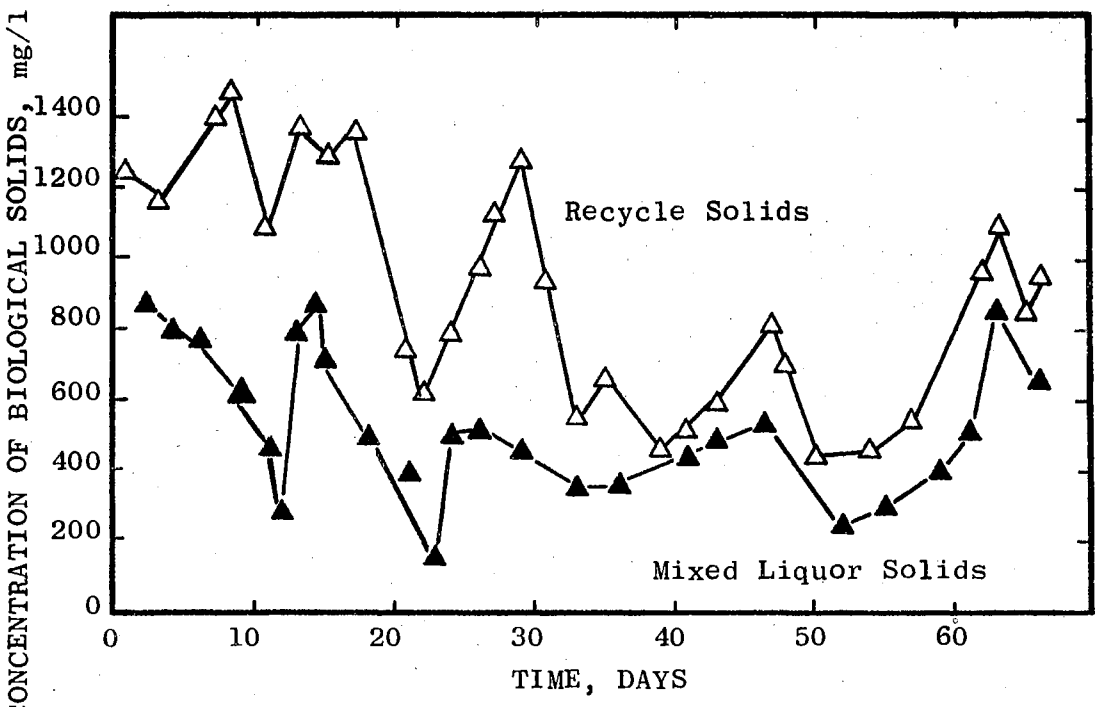

Figure 90A. Variation in the Conc. of Biological Solids during Steady State Operation with Recirculation of Sludge. (S_i = 1000 mg/l; D = 1/2 per Hour; a = 0.25)

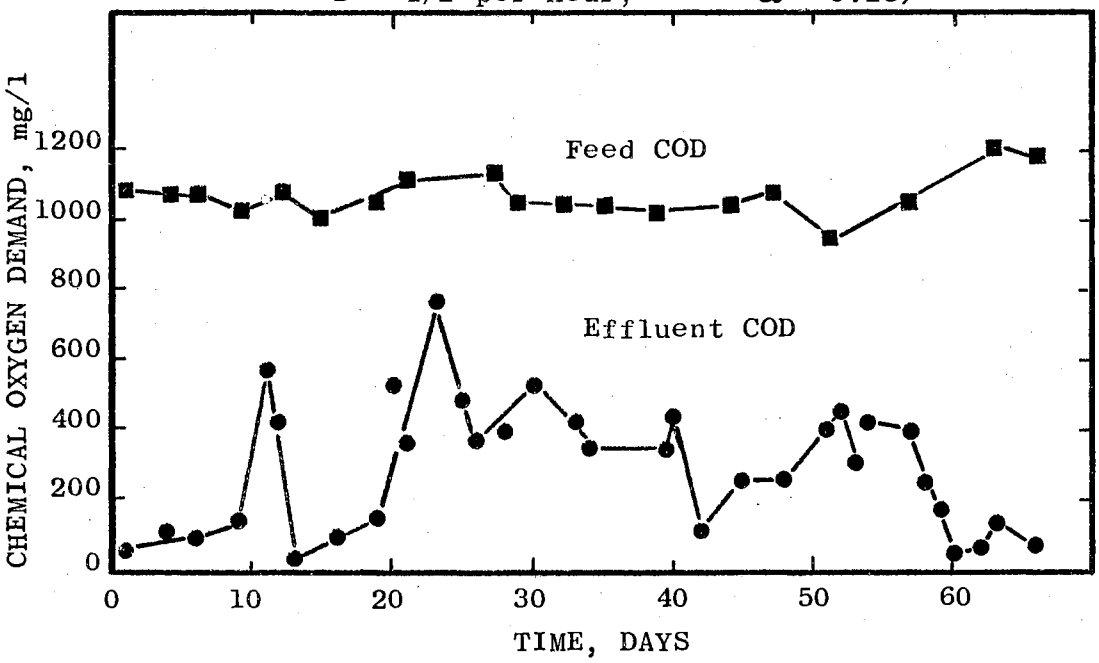

Figure 90B. Variation in the Conc. of COD during Steady State Operation with Recirculation. (S_i = 1000 mg/l; D = 1/2 $Hour^{-1}$; a = 0.25)

TABLE VII

STATISTICAL ESTIMATION OF THE STEADY STATE PARAMETERS AT DIFFERENT DILUTION RATES

(S_i = 1000 mg/l Glucose; With Recirculation)
(α = 0.25; c = 1.50)

Mean Residence Time ($\bar{t}$)	$\bar{x}$	$c\bar{x}$	$\bar{S}_i$	$\bar{S}$	$\bar{S}_c$	Coefficient of Variance				$\frac{\bar{S}_i - \bar{S}}{\bar{x} \cdot \bar{t}}$	Loading Factor lbs COD/ lbs SS/day	Effy.	Rate of Output of Cells mg/l/hr
						$\bar{x}$	$c\bar{x}$	$\bar{S}$	$\bar{S}_c$				
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
6	837	1295	1040	60	6.40	21.9	27.8	36.0	87.50	0.195	9.94	94.20	122
4	797	1236	1060	87	15.10	32.4	44.7	36.0	56.80	0.305	15.97	91.70	174
3	753	1121	1067	79	16.60	31.2	48.8	56.1	117.50	0.438	22.65	92.60	219
2	500	861	1065	277	156.00	39.3	48.0	66.7	111.50	0.788	51.10	74.00	218

that COD loadings of up to 22.65 lbs. per lb. of biological solids can be applied with efficiencies greater than 91.70 per cent.

(d) Studies with Total Recirculation of Sludge

One experiment was conducted with total recirculation of sludge in order to investigate whether a steady-state could be attained. Figure 91 shows the variations in biological solids concentration and in COD. It can be seen from the figure that the concentration of biological solids increased from 400 mg/l at the start, to 3600 mg/l after 12 days of operation, and then decreased again to 2000 mg/l. Continued operation indicated that there was a cyclic variation in the concentration of biological solids between 2000 mg/l and 3600 mg/l; the variations in effluent COD were less severe (between 50 and 90 mg/l). Determination of percent volatile solids indicated that this parameter remained constant with a mean value of 86.9 per cent. The lowest value obtained was 84 per cent, and the highest value was 91.75 per cent. During the 30 days of operation there was no decrease in volatile suspended solids content as had been reported by other research workers in the case of total oxidation systems (110, 113), and it was possible to operate the system without sludge wasting.

5. Effect of Dilution Rate on Steady-State Parameters

In order to study the effect of dilution rate on the various parameters for assessing the behavior of the sys-

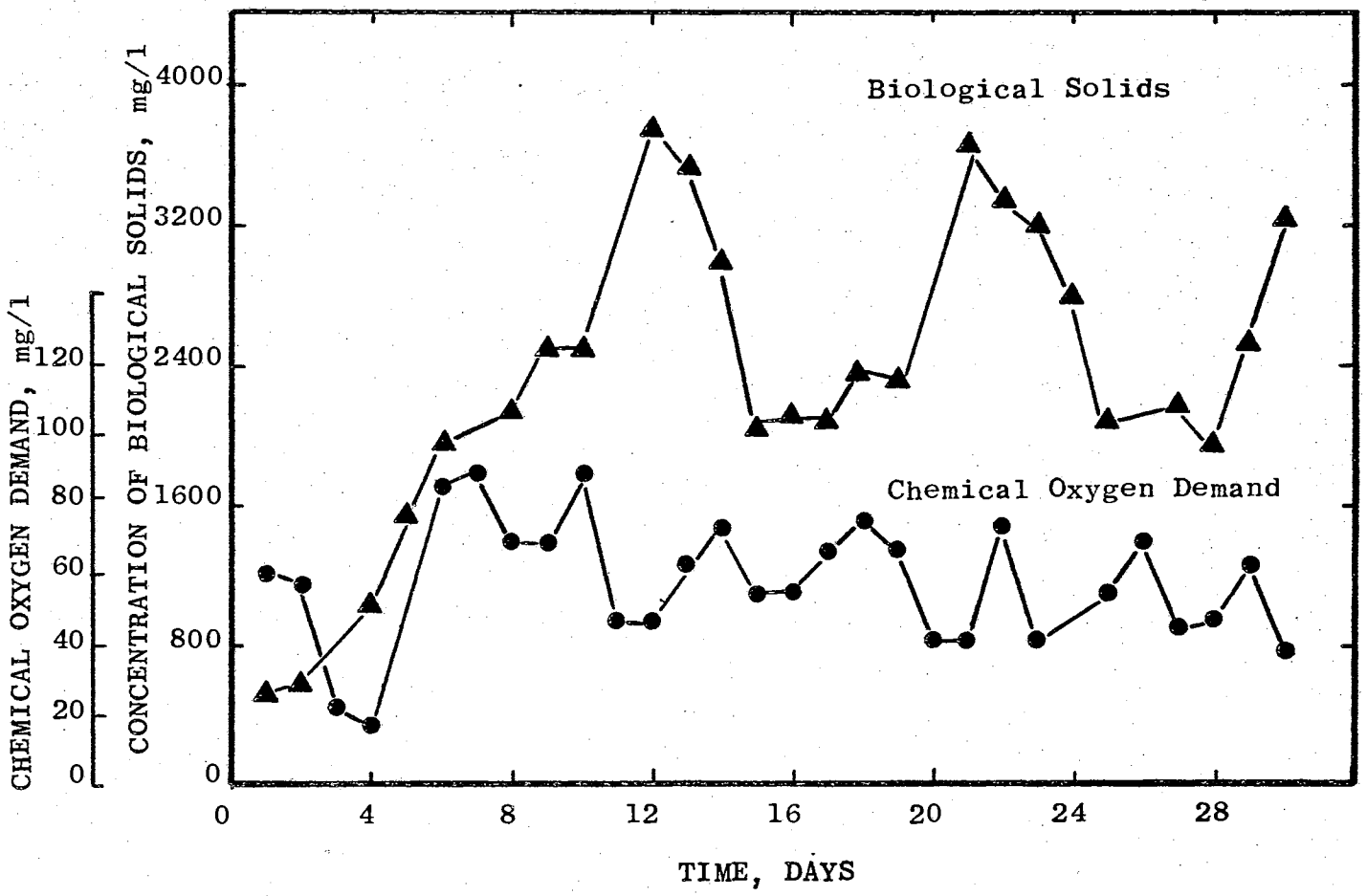

Figure 91. Variation in MLSS and COD with Total Recirculation of Sludge. ($\bar{t}$ = 4 Hours; S_i = 1000 mg/l Glucose)

tem, the experimental values of steady-state parameters were compared with theoretical values calculated according to the theory of Herbert, et al. (5). Figure 92 shows the variations in biological solids, effluent COD, and carbohydrate at different dilution rates with an inflow substrate concentration of 1000 mg/l glucose. It can be seen from the graph that the experimental values of biological solids are higher than the theoretical values calculated using the growth parameters obtained by batch experiments. The theoretical values were calculated in two ways: (a) from the values of μ_m, k_s, and Y_B obtained separately for each dilution rate, and (b) from the mean values of μ_m, k_s, and Y_B for all dilution rates. The calculated values of steady-state parameters are shown in Table VIII. The variations in observed values of COD and carbohydrate concentrations follow a similar pattern; however, when the variations in experimental values are compared with the theoretical values, there is a difference in the shape of the curve particularly during the dilute-out portion. A similar pattern was obtained during studies at an inflow substrate concentration of 3000 mg/l; these results are shown in Figure 93. Table IX shows the theoretical values calculated according to the theory of Herbert, et al. (5). An additional method of obtaining an estimate of the predicted dilute-out curve was employed. The mean value of yield coefficient from the continuous flow experi-

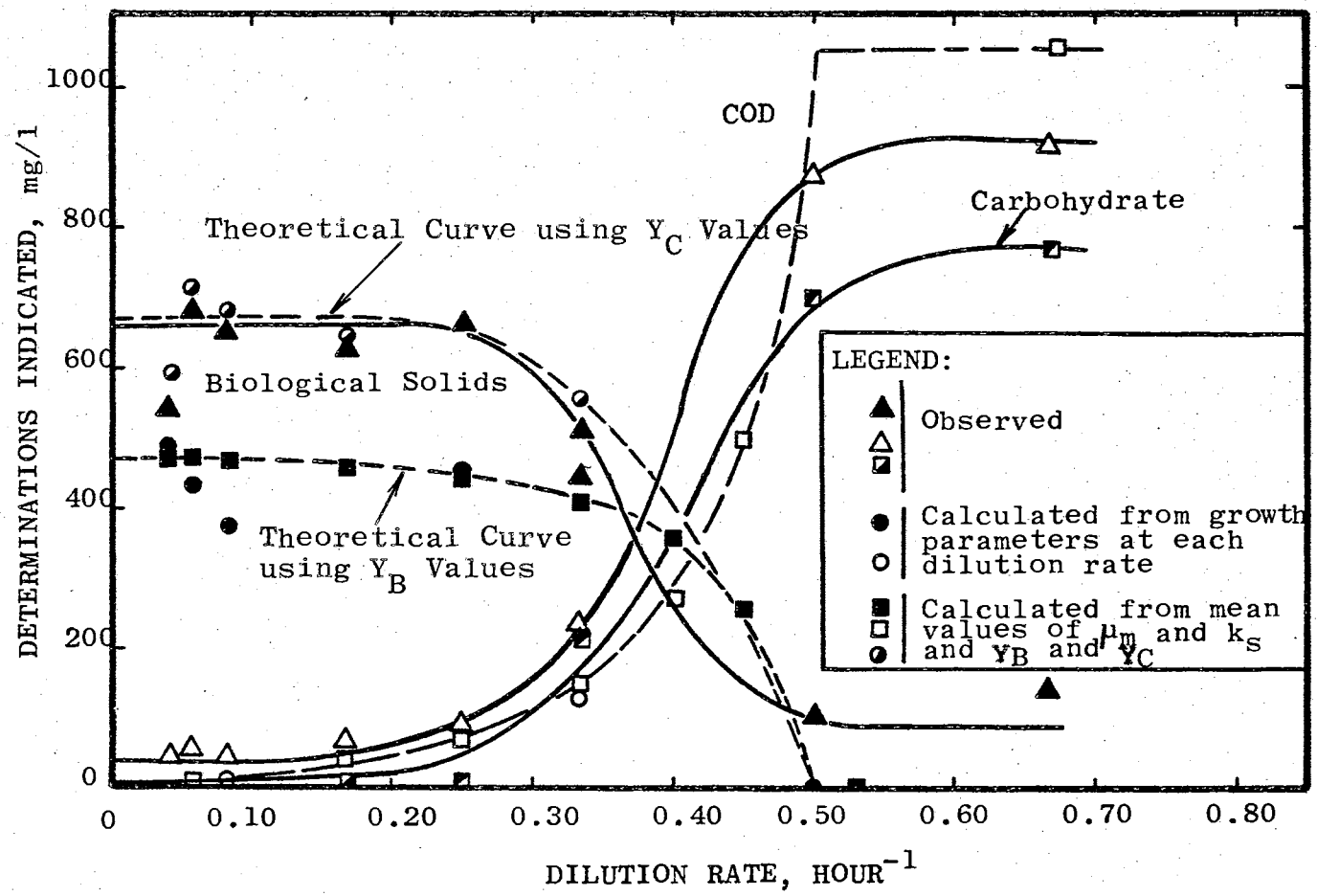

Figure 92. Dilute-Out Curves for Biological Solids, and Substrate with S_i = 1000 mg/l Glucose. (Without Recirculation)

TABLE VIII

STEADY-STATE VALUES CALCULATED FROM GROWTH PARAMETERS DETERMINED AT EACH DILUTION RATE BY BATCH EXPERIMENTS

(Si = 1000 mg/l Glucose; No Recirculation)

Dilution Rate Hr^{-1}	μ_m Hr^{-1}	k_s	Y_B	S_i mg/l	$\bar{S}$ mg/l	$\bar{x}$ mg/l	$\bar{S}_1$ mg/l	$\bar{x}_1$ mg/l	Y_C	$\bar{S}_2$	$\bar{x}_2$
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
1/24	0.390	64	0.46	1080	7.7	493.2	7.6	473	0.552	7.65	592
1/18	0.367	89	0.42	1047	16.0	433.0	10.5	471	0.692	10.50	716
1/12	0.567	86	0.37	1025	14.7	373.7	16.5	469	0.675	16.50	680
1/6	0.645	123	0.46	1058	42.9	466.9	40.8	458	0.636	40.80	646
1/4	0.513	81	0.46	1057	77.0	450.8	79.2	440	0.685	79.10	670
1/3	0.683	138	0.48	1051	131.4	441.4	148.3	409	0.615	149.50	554
1/2	0.540	98	0.48	1057	1225	-	1435	-	0.558	1057	0
1/1.50	0.545	35	0.48	1091	1091	0	1058.0	-	0.785	1091	0
Mean	0.531	89	0.45	1058							

Note: $\bar{S}$ and $\bar{x}$ are calculated from growth parameters obtained at each dilution rate from batch experiments

$\bar{S}_1$ and $\bar{x}_1$ are calculated from the mean values of growth parameters obtained from batch experiments

$\bar{S}_2$ and $\bar{x}_2$ are calculated from the mean values of μ_m and k_s but with Y_C

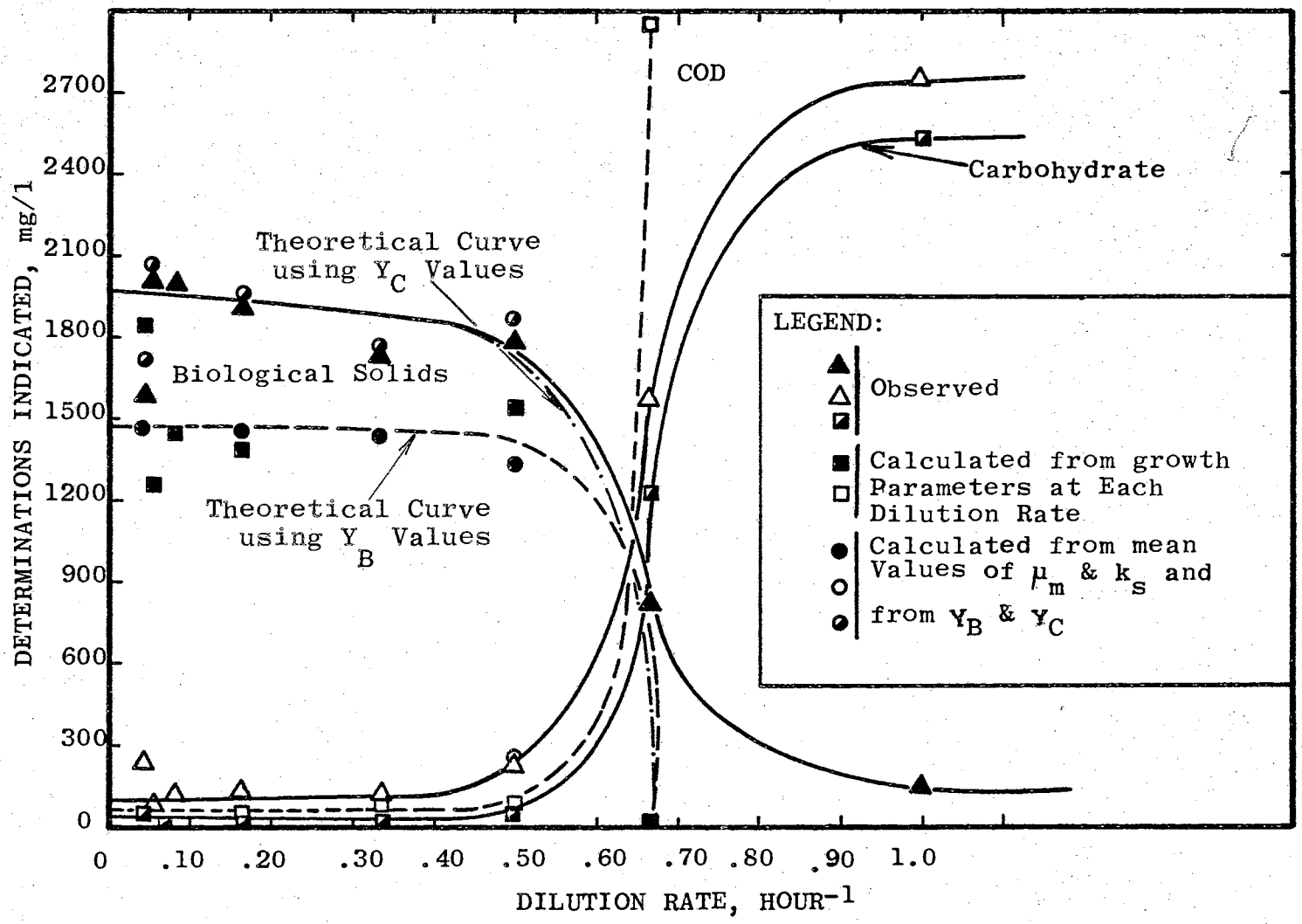

Figure 93. Dilute-out Curves for Biological Solids and Substrate with S_i = 3000 mg/l Glucose. (Without Recirculation)

TABLE IX

STEADY-STATE VALUES CALCULATED FROM GROWTH PARAMETERS DETERMINED AT EACH DILUTION RATE BY BATCH EXPERIMENTS

(S_i = 3000 mg/l Glucose; No Recirculation)

Dilution Rate	μ_m	k_s	Y		$\bar{S}$	$\bar{x}$	$\bar{S}_1$	$\bar{x}_1$	$\bar{S}_2$	$\bar{x}_2$	$\bar{S}_i$
Hr^{-1}	Hr^{-1}		Batch	Cont.	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
1/24	0.430	45	0.606	0.562	4.88	1845	4.25	1460	4.88	1710	3048
1/18	0.390	92	0.406	0.664	15.40	1260	5.81	1460	15.40	2060	3121
1/12	0.658	85	0.470	0.67	12.30	1442	9.05	1460	12.30	2055	3086
1/6	0.520	113	0.450	0.633	53.50	1390	21.60	1452	53.50	1955	3147
1/3	0.453	29	0.560	0.572	80.50	1710	68.50	1430	80.50	1750	3139
1/2	0.633	22	0.500	0.608	82.80	1540	252	1342	82.80	1870	3163
1/1.50	0.588	29	0.440	0.430	2950	0	3089	0	2950	0	2950
1	0.445	51	0.362	0.365	3080	0	3089	0	3080	0	3080

Note: $\bar{S}$ and $\bar{x}$ are calculated from growth parameters obtained from batch experiments for each dilution rate separately

$\bar{S}_2$ and $\bar{x}_2$ are calculated the same as $\bar{S}$ and $\bar{x}$, but the yield value of continuous-flow experiment (column 5) is used

$\bar{S}_1$ and $\bar{x}_1$ are calculated from the average values of μ_m, k_s, and Y_B

ments was used, together with the mean values of μ_m and k_s obtained by batch experiments to calculate the theoretical values of $\bar{S}_2$ and $\bar{x}_2$. These values are given in Table VIII and IX, and are plotted in Figures 92 and 93. Table X shows the theoretical values of steady-state parameters calculated from the values of μ_m, k_s, and Y_B for each dilution rate separately for studies with recirculation. Also given in the table are the theoretical values of $\bar{S}$ and $\bar{x}$ calculated from the mean values of μ_m, k_s and Y_B. When these values are compared with the experimental values in Table VII, it can be seen that the experimental values of biological solids are always higher than the theoretical values. This is also reflected in the values of yield coefficients, calculated from the continuous-flow experiments shown in the last column of Table X.

Figures 94 and 95 show the theoretical values of steady-state parameters calculated with the yield values obtained according to the theory of Hetling, et al. (15). The values of yield coefficients were taken from Figures 66 and 67 representing continuous-flow experimental data. It can be seen from the figures that the dilute-out curves for biological solids and substrate concentration are similar to the other theoretical curves presented earlier. Also shown in Figures 94 and 95 are the values of μ_m obtained from batch experiments. The variations in this parameter with changing dilution rate have already been

TABLE X

STEADY-STATE VALUES CALCULATED FROM GROWTH PARAMETERS DETERMINED AT EACH DILUTION RATE BY BATCH EXPERIMENTS

(S_i = 1000 mg/l Glucose; With Recirculation)
(α = 0.25; c = 1.50)

Mean Residence Time ($\bar{t}$), Hr.	μ_m Hr^{-1}	k_s	Y_B	$\bar{S}_i$ mg/l	$\bar{S}$ mg/l	$\bar{x}$ mg/l	$\bar{S}_1$ mg/l	$\bar{x}_1$ mg/l	Y_C
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
6	0.40	59	0.374	1040	42	427	52	561	0.748
4	0.70	100	0.584	1060	33	685	55	569	0.716
3	0.47	178	0.590	1067	176	600	90	555	0.666
2	0.46	87	0.440	1065	277	396	232	473	0.555

Note: $\bar{S}$ and $\bar{x}$ are calculated using μ_m, k_s, and Y_B for each dilution rate separately

$\bar{S}_1$ and $\bar{x}_1$ are calculated using mean values of μ_m, k_s, and Y_B

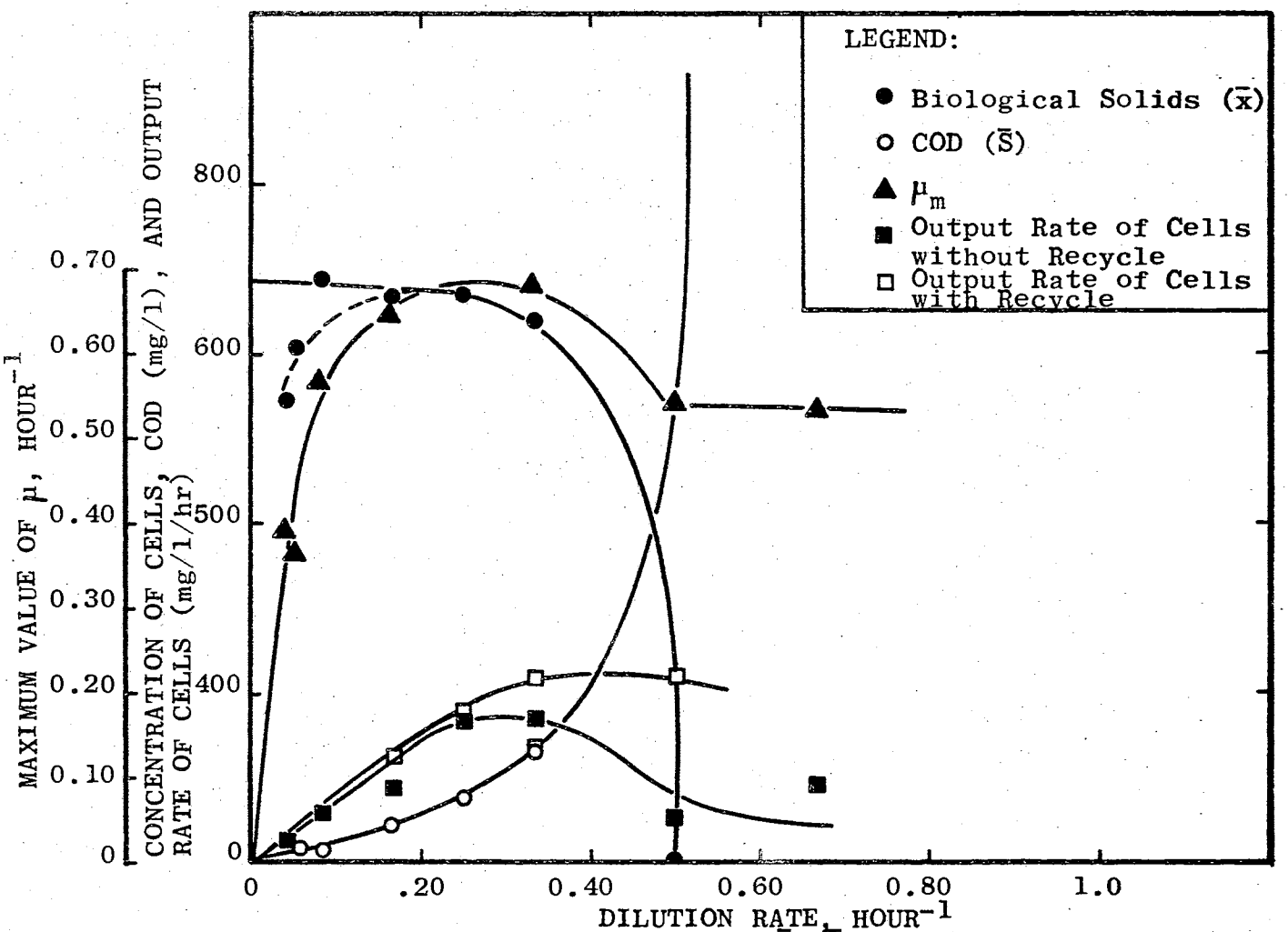

Figure 94. Effect of Dilution Rate on $\bar{x}$, $\bar{S}$, μ_m and Output Rate of Cells when S_i = 1000 mg/l Glucose. ($\bar{x}$ and $\bar{S}$ calculated from Y Values obtained according to the Theory of Hetling, et al.)

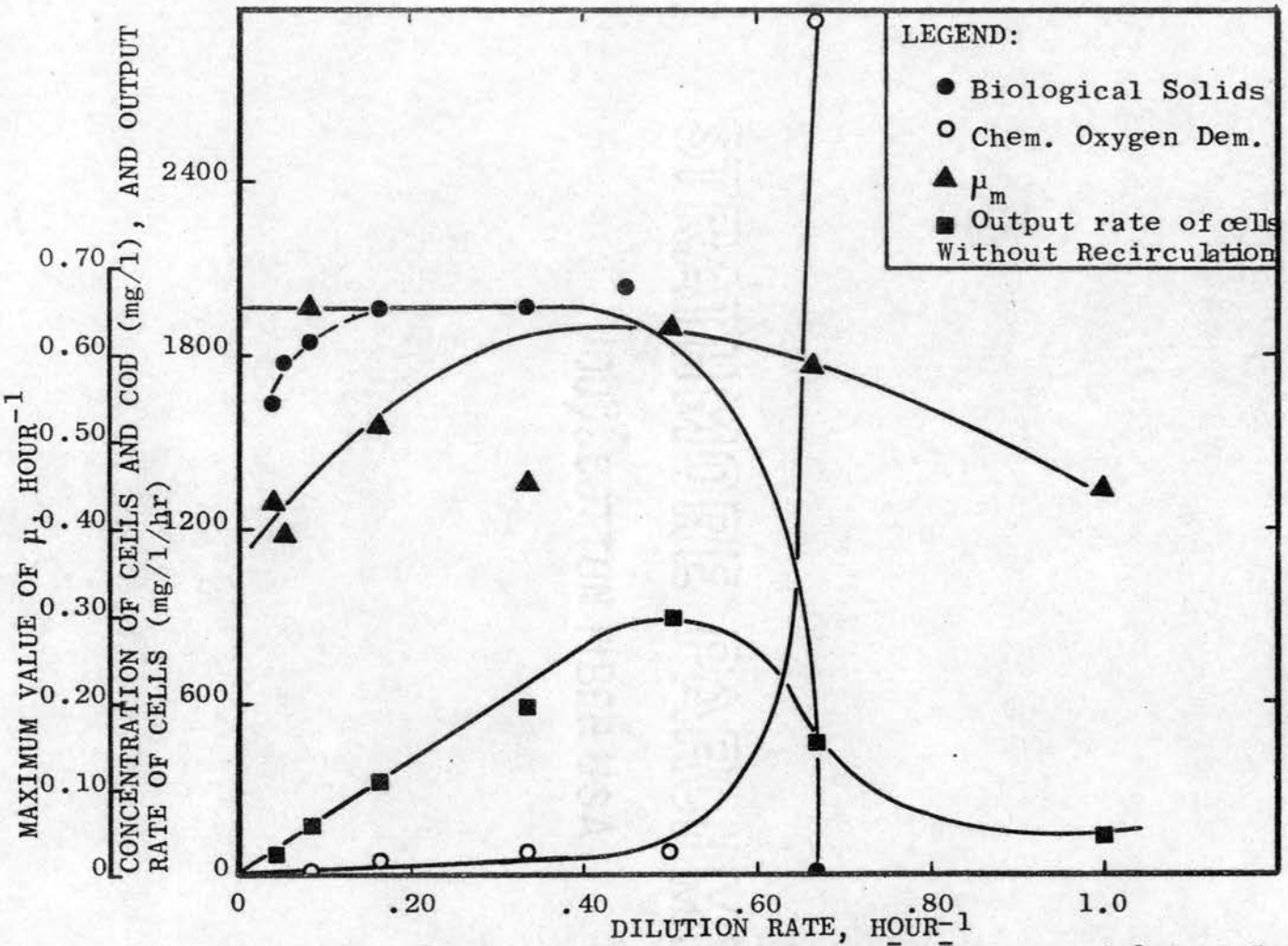

Figure 95. Effect of Dilution Rate on $\bar{x}$, $\bar{S}$, μ_m, and Output Rate of Cells when S_i = 3000 mg/l Glucose. ($\bar{x}$ and $\bar{S}$ calculated from Y Values obtained according to the Theory of Hetling, et al.)

discussed. The rates of output of cells are also plotted in Figures 94 and 95. The output rate increased with dilution rate until the washout of cells occurred in the system; it decreased at higher values of dilution rate. However, when recirculation of sludge was employed, the output rate of cells did not decrease at the same rate as without recirculation. It appears that the recirculation of sludge increased the output rate of cells at identical dilution rates. When the inflow substrate concentration was increased to 3000 mg/l glucose, the output rate of cells also increased for the same dilution rate. In Figure 95 is shown the output rate of cells when S_i = 3000 mg/l glucose. These results agree with the theoretical discussions presented by Herbert (4).

Figure 96 shows the relationship between specific substrate assimilation rate and dilution rate. The specific substrate assimilation rates were calculated from the mean residence time and steady-state concentrations of substrate and biological solids. Two different straight lines were obtained, one for the system without recirculation and another for the system with recirculation of sludge. These relationships can be represented by the following equations:

$$s_r = \frac{D(S_i - \bar{S})}{\bar{x}} = 1.58\ D \qquad (98)$$

for studies without recirculation, and

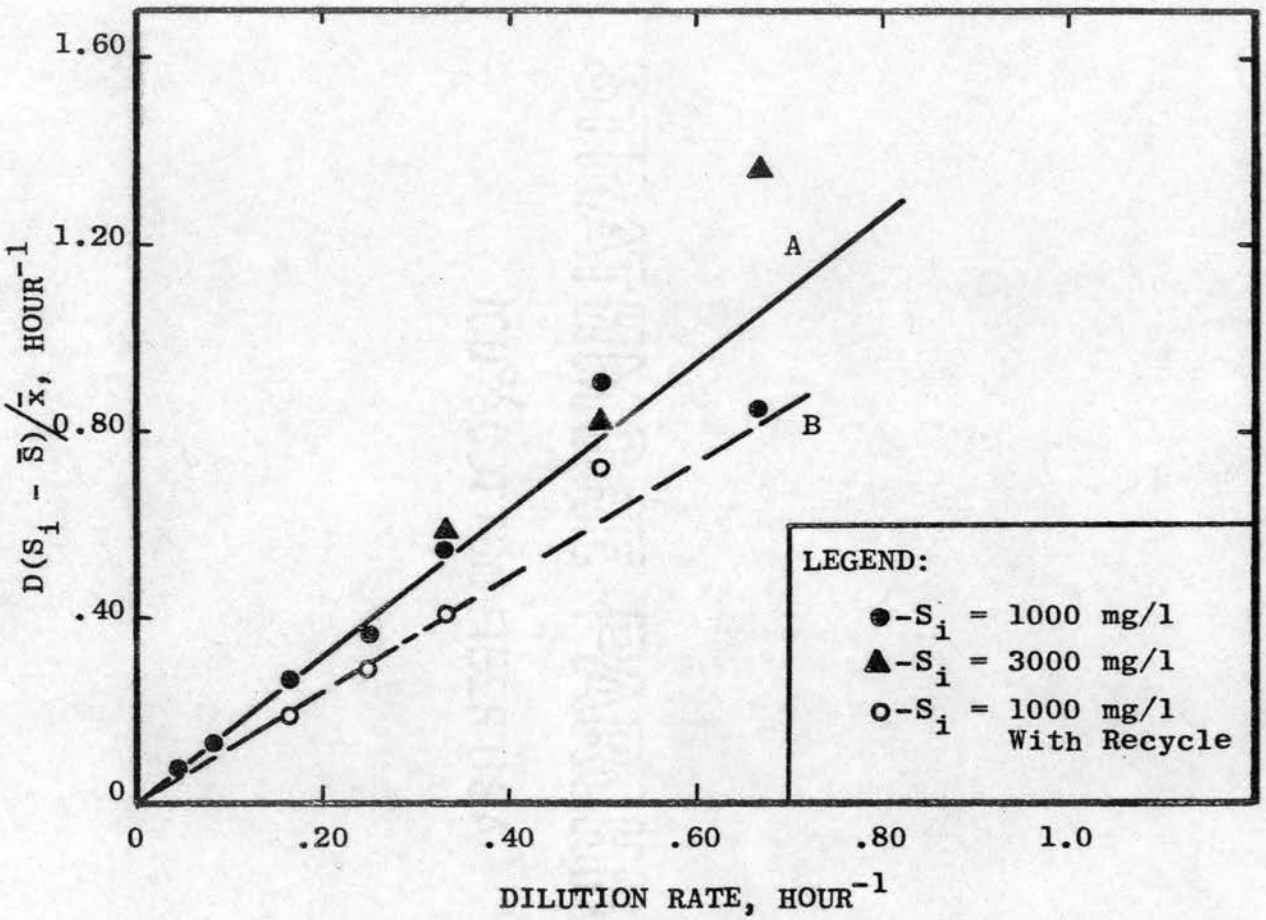

Figure 96. Relationship between Dilution Rate and Specific Substrate Utilization Rate.
A - Without Recirculation; B - With Recirculation.

$$s_r = 1.225\ D \qquad (99)$$

for studies with recirculation. It can be seen from Figure 96 that so far as substrate removal is concerned, the system without recirculation is more efficient than with recirculation. Also, the specific substrate removal rate increases with increasing dilution rate, indicating an increase in the activity of the organisms at higher dilution rates.

The various results obtained in the present research work have been presented in this section. The validity of relationships and formulations employed and their applications will be discussed in the following chapter.

CHAPTER VI

DISCUSSION OF RESULTS

1. Relationship between Substrate Concentration and Rate of Growth

The accurate prediction of the performance of a continuous flow, completely-mixed activated sludge process depends upon the establishment of the kinetic relationships between substrate consumption and growth. It is evident from theoretical considerations of the operation of steady-state completely-mixed systems that the microorganisms or sludge must multiply or increase exponentially in the reactor; therefore, the expression relating rate of growth and concentration of biological solids is of first order, and can be represented by Equation 8. Monod (20) has shown that the specific growth rate (μ) is not a constant and varies with the concentration of growth-limiting factor. Hinshelwood (53) has shown that the exhaustion of nutrients is the factor limiting growth. McKinney (78) has also indicated that substrate-limiting conditions prevail in the continuous-flow completely-mixed system. McKinney has considered the rate of increase of biological solids as a function of substrate concentra-

tion, and presented equations to relate rate of growth and substrate concentration. However, it can be shown that McKinney's relationships are the same as Monod's, since the growth of microorganisms and substrate utilization are related by the yield coefficient, and the growth constants differ only by this factor.

Keshavan, et al. (87) have presented a second order equation to describe bacterial growth in a completely-mixed reactor. Since the above theory does not support the exponential growth of microorganisms in a completely-mixed reactor it also does not support the steady-state characteristics of the system. The results presented in the previous chapter clearly indicate that the specific growth rate varies with the concentration of growth-limiting factor, and that the variations can be closely represented by the equation proposed by Monod (20). Further evidence to support this equation was presented by Wilson (80). Wilson compared the kinetic theories proposed by Garrett and Sawyer (18) and adopted by Eckenfelder (84) with the Monod relationships. He concluded that Monod's expression for specific growth rate (μ) is more suitable than the expressions proposed by Garrett and Sawyer. The above conclusions were based on experiments conducted with activated sludge treating a mixed chemical waste. From these studies and those reported by Novick and Szilard (6), and Herbert, et al. (5) it is fairly evident that microbial growth can be repre-

sented by Equation 14. In the present study, experiments were designed specifically to test the validity of Monod's relationship, and the results leave little doubt that there is a continuous function which describes the relationship between the log growth-rate constant and concentration of limiting nutrient.

Fujimoto (81) considered the specific growth rate to be a function of both substrate concentration and solids concentration. According to him, Equations 75 and 76 can be used to predict the entire course of bacterial growth and substrate removal. These equations could not be verified by the experimental results obtained in this research, since only very low initial concentrations of biological solids (10 to 20 mg/l) were used in the batch experiments to determine exponential growth rate (μ), and it is believed that this low solids level will not have any effect on the determination of μ. Boranzi, et al. (72) have also presented equations in which the rate of substrate removal $-\left|\frac{dS}{dt}\right|$ is considered to be a function of both substrate and solids concentration. These equations were also not verified in the present work, since the substrate removal rates were not followed in the batch experiments conducted to determine the physiological growth parameters.

Contois (71) also felt that the specific growth rate is not only a function of substrate concentration, but is also a function of the concentration of biological solids.

Even though the consideration of substrate and solids concentration seems to be logical in the expression describing growth, the present research indicates that the specific growth rate can be described adequately as a function of substrate concentration alone by Equation 14. Figures 14 to 52 lend support to the suitability of Monod's empirical equation for describing bacterial growth. Although Equation 14 is empirical, it is of the same form as the Michaelis-Menten equation and growth occurs as a result of a series of enzyme reactions of which one might control the overall process. Neither the expressions of Schulze (90) nor that of Moser (19) fit the present experimental data (Figures 30 to 33, and 50 to 52).

Weston and Stack (85) considered that the completely-mixed activated sludge process is operating in a phase similar to the stationary phase in batch culture. From the previous discussions, it can be seen that the completely-mixed system cannot operate at the stationary phase. Since the establishment of steady state is an inherent characteristic of a continuous flow completely-mixed system, the organisms have to be multiplying exponentially during steady state operation. If they were in the stationary phase at which the rate of growth is equal to the rate of death, the hydraulic flow rate through the reactor would result in the gradual washout of the culture. In such a system, the dilute-out of the culture

can be prevented only by recirculation of sludge to balance the washout. The present study indicates that "steady-state" operations are possible even without recirculation of sludge, and therefore the theory proposed by Weston and Stack (85) does not seem to be applicable for completely-mixed continuous flow systems operating under "steady-state" conditions.

2. Relationship between Substrate Consumption and Growth

Most early research workers were of the opinion that there is always a constant relationship between growth and substrate consumption. Monod (20), Herbert, et al. (5), and Novick and Szilard (6) have reported constant yield coefficients and have used this constant value in expressions describing steady-state kinetics. They employed pure cultures of microorganisms utilizing a single substrate. This probably led other research workers, employing mixed cultures, to believe that the yield coefficient is a constant. Garrett and Sawyer (18), Helmers, et al. (61), and Gellman and Heukelekian (62) have reported yield coefficients between 0.50 and 0.60 for activated sludge utilizing different industrial wastes. These studies probably influenced Servizi and Bogan (64) in their attempt to relate chemical oxygen demand, free energy of oxidation, and ATP yield to yield coefficient. Kashavan, et al. (87) have also assumed a constant value for the yield coefficient to predict the performance of continuous flow systems.

Marr, et al. (89) realized that the yield coefficient may not be a constant even for pure cultures utilizing a single organic compound. They have explained this behavior as due to a maintenance requirement for exogenous substrate by the culture. Schulze (90) also reported a change in yield coefficient from 0.44 to 0.54 when the dilution rate (D) was increased from 0.06 to 0.66 per hour. A pure culture of Escherichia coli utilizing glucose was used in the above studies. Schulze explained this as due to varying amounts of endogenous respiration. Hetling, et al. (15) have reported, from continuous flow experiments with pure cultures of Pseudomonas fluorescens, Escherichia coli, Alcaligenes faecalis, and Bacillus cereus, that significant variations in yield coefficient may be attributed to the maintenance requirement and the presence of dead cells in the system. These variations in yield coefficient become more complicated in the case of mixed cultures since the metabolic pathways will be different for different organisms. Rao and Gaudy (16) have also reported varying yield coefficients for activated sludge utilizing glucose as substrate.

The results presented in the previous chapter indicate that the yield coefficients change with dilution rate. Also, the yield coefficients calculated from batch experiments were always lower than those in the continuous

flow systems. This may be explained as due to changes in the environmental conditions under which the yield determinations were made. Therefore, using the values of yield from batch studies to predict the performance of continuous flow systems may give erroneous results. However, the variations in Y_B and Y_C are similar with respect to dilution rate (Figures 66 and 67). Comparison of theories proposed by Marr, et al. (89) and Hetling, et al. (15) indicates that the theory proposed by the latter agrees with the present results more closely than that of the former. In the dilute-out portions of the curve (Figure 65), the experimental data do not fit a straight line relationship. It may be concluded from these results that variation in yield coefficient with dilution rate is caused by two factors, namely, (a) substrate consumption for maintenance, and (b) effect of death rate of cells in the measurement of yield coefficient.

Examination of the steady-state concentration of biological solids at each dilution rate indicates the presence of "oscillation." Since the variations in substrate concentration are negligible compared to the variations in biological solids concentration, this may also be attributed to the changing yield coefficients. The change in yield coefficient during "steady-state" operation at a particular dilution rate may be explained as due to chang-

ing predominance of species in the population. Evidence for this hypothesis can be obtained from the works of Rao and Gaudy (16). They have reported a variation in yield coefficient from 0.48 to 0.82 in batch studies, and they have attributed this to observed changes in predominance. Therefore, it can be concluded that the change in yield coefficient during "steady-state" operation may be attributed to predominance or selection, whereas variation in yield coefficient at different dilution rates may be due to (a) maintenance requirement, (b) active fraction of the bio-mass measured by the death rate of cells, and (c) predominance or selection of species. Since the separate determination of death rate of cells and maintenance requirement is difficult, Hetling, et al. (15) have referred to this as "heterogeneous metabolic rate." In the present study, microscopic examination of the biological population at different dilution rates revealed that the population characteristics change with dilution rate, particularly at high dilution rates ($D > 0.25$ per hour). Absence of predatory organisms were observed at dilution rates greater than 0.25 per hour. Individual identification of the species at different dilution rates was not possible, since the original inoculum was obtained from the primary effluent of the local waste treatment plant, which contains a wide variety of organisms. Further, it is beyond the scope of this research to study the population dynamics of a continuous-flow culture.

3. Steady State Kinetics of Continuous Flow Completely-Mixed Process

The discussion so far presented indicates that the physiological growth parameters μ_m, k_s, and Y can be determined from batch experiments conducted with cultures maintained at steady-state. It is the purpose of this research to investigate whether these parameters can be used to predict the performance of the continuous flow activated sludge process. It can be seen from Figures 92 and 93 that theoretical curves obtained either with mean values of growth parameters or with individual values of growth parameters give identical dilute-out curves. However, when the yield coefficient from continuous culture (Y_C) is used with the batch values of μ_m and k_s to predict the steady state levels of substrate and cells, the theoretical curve and experimental data were identical until "dilute-out" began in the system. When the dilute-out starts in the system, the theoretical curves drop at a faster rate than the experimental values. A tailing off of the experimental values seems to be present in the system. A similar result has been reported by Herbert for pure culture studies of Aerobacter cloacae. This indicates that the value of maximum growth rate (μ_m) determined from batch experiments must be lower than that of continuous cultures, or that washout rate is lower than would be predicted from Equation 19. The latter

explanation seems to be more appropriate than the former, since even in the case of dye experiments a difference was observed between theoretical and observed values of dye concentration (Figure 11). It could be expected that this effect would be more significant in the case of suspended solids than in the case of soluble materials. The dilute-in of substrate also exhibited a similar difference between the theoretical and experimental values.

In order to determine whether the differences between effective yield coefficient (Y) and true yield coefficient (Y_t) might be related to the tailing-out effect noted above, theoretical values of steady state parameters were calculated according to the theory proposed by Hetling, et al. (15). These are shown in Figures 94 and 95. It can be seen from these figures that the theoretical curves obtained by all of the methods are identical, and no tailing-off of the washout curve is observed. It can be concluded from the above discussion that the kinetic relationships presented in the previous chapters are applicable only before the washout of the system begins.

4. Maintenance Requirement of Activated Sludge

The changing yield coefficients at different dilution rates have been attributed to the maintenance requirement by Marr, et al. (89). Figure 65 shows the variations in $1/\bar{x}$ with mean residence time. The experimental values do not follow the straight line rela-

tionship for the entire range of dilution rate studied. This may be due to the fact that the concentration of biological solids was plotted instead of yield coefficient. It would appear that the yield coefficient is a more proper measure of maintenance requirement than cell concentration. This is evident from Figures 66 and 67, wherein the reciprocals of the yield coefficient are plotted against mean residence time. The specific maintenance rates obtained from these graphs (0.019 and 0.02 per hour) agree with the values obtained by Marr, et al. (89), and by Hetling, et al. (15). The latter workers have obtained values of 0.028 per hour and 0.01 to 0.06 per hour, respectively, for pure culture experiments. Schulze (90) has reported that the changing yield coefficients are due to varying rates of endogenous respiration; however, Stewart, et al. (21) and McKinney (78) have reported that the specific endogenous respiration rate is a constant and does not vary either in the presence or absence of substrate. Further evidence to this can be obtained from the works of Cochrane and Gibbs (117), who grew washed cells of Streptomyces coelicolor, that had initially been cultured on a C^{14} labeled glucose medium, on unlabeled glucose and pyruvate. They have shown that the endogenous respiration, as indicated by the total radioactivity of respired CO_2, was the same as when the washed cells were allowed to

to respire in the absence of substrate. This indicates that the endogenous respiration rate can be considered as a constant proportion of the cell mass and is not affected by the concentration of substrate present. Therefore, it can be concluded that maintenance requirement may be the factor affecting the yield coefficient.

5. Phenomenon of Oscillation in Continuous Culture

Fluctuations in the concentration of biological solids have been observed by almost all research workers. Kincannon (23), on the basis of microscopic observation, has attributed this to changes in predominance or selection of species. From the figures presented in the previous chapter, it can be seen that fluctuations are present at all the dilution rates studied. Sack and Schulze (52) have also observed daily fluctuations in solids concentration, and they have attributed this to the differences between growth rate and washout of culture from the reactor. Since it was found in the present study that fluctuations are inevitable in a continuous-flow culturing device, a statistical estimation of the population mean was obtained from steady-state parameters. This seems to agree with the theoretical values provided the yield coefficient is also taken from continuous-flow experiments.

In order to verify that the daily fluctuations in the concentration of biological solids are not due to

experimental error, hourly samples were taken in a few of the experiments, and they have been found to be nearly constant. One such set of data is shown in Table XI. Examination of the values in Table XI leaves little doubt that the daily fluctuations are natural phenomena occurring in any continuous culture device. It was not possible to ascertain by microscopic examination that the fluctuations are caused by changes in predominance of species, because of the wide variety of organisms present in the inoculum used for the culture. Since the physical parameters of the systems, such as temperature, pH, and dissolved oxygen, were controlled, it may be concluded that the fluctuations are caused either by predominance or by changing yield coefficients. However, it is important to point out that oscillations have been observed even in pure culture experiments conducted under controlled conditions (15, 52, and 90), and the "steady-state" has been referred to as one of the "pseudo equilibrium." It appears from the above discussion that fluctuations are inevitable in a completely-mixed biological system, and the factors that may influence this behavior are: (a) yield coefficient, (b) environmental conditions, (c) predominance or selection of species, and (d) flocculation and wall growth. Since some of the above factors are uncontrollable, it is appropriate to collect sufficient data and then statistically estimate the "steady-state" parameters.

TABLE XI

HOURLY VARIATIONS IN "STEADY-STATE" PARAMETERS

(S_i = 1000 mg/l Glucose; D = 1/6 Hr^{-1})

(No Recirculation)

Time Hr	Biological Solids mg/l	COD mg/l	Carbohydrate mg/l
(1)	(2)	(3)	(4)
16:30	651	76.60	9.30
18:00	616	72.60	6.20
19:00	593	80.50	5.43
20:00	715	80.50	6.40
21:00	524	76.60	6.50
23:00	532	80.50	8.20

6. Rate of Substrate Consumption

Figure 96 shows the relationship between rate of substrate consumption per unit weight of solids and dilution rate. It can be seen that the straight line relationship is independent of inflow substrate concentration. A single straight line represents the substrate removal rate for both values of S_i (1000 and 3000 mg/l) without recirculation of sludge. The specific substrate removal rate increases with dilution rate indicating a higher rate of substrate removal at higher values of D. This indicates that the organisms are more active at higher dilution rates than at lower values of D. This is also reflected in the specific growth rate values obtained from batch experiments. In Figure 96 is also shown the specific substrate removal curve for studies with recirculation with

an inflow substrate concentration of 1000 mg/l. It appears from this graph that when recirculation of sludge is employed, the specific substrate removal rate decreases, indicating a lower activity of the cells than in the case of studies without recirculation. This may be due to the fact that when sludge is recycled the biological solids concentration in the reactor is at a higher level than in the absence of sludge recycle for a particular mean residence time. Also, the rate of addition of substrate or the loading factor is reduced in the case of studies with recirculation due to the addition of return sludge. Therefore, the substrate available per unit weight of cells is reduced for a given inflow rate of substrate, which might reduce the activity of biological solids.

The above discussion indicates that the specific substrate consumption rate can be used for design purposes, together with growth parameters. From the efficiency of treatment required, the substrate concentration in the reactor can be calculated. Then, assuming a particular mean residence time, the specific substrate utilization rate can be obtained from Figure 96. From the specific substrate utilization rate, the biological solids concentration in the reactor and the output rate of cells can be calculated.

7. Unit Activity of Microbial Populations in a Completely-Mixed Reactor

Moser (19) has indicated that the assumption made by Herbert, et al. (5) of 100 per cent viability of population in a continuous-flow reactor is not correct. This probably has led McKinnery (78) and Hetling, et al. (15) to introduce a nonviability factor in their mathematical derivations. They have differentiated between the active cell mass and inactive cell mass by including a factor for active fraction. Wuhrmann (66) has proposed a method to evaluate the active and inactive fraction of a biomass. The method involves the measurement of oxygen consumed per unit weight of washed cells per unit time. Since only active fraction of the biomass will require oxygen, unit activity might be taken as a measure of the active fraction. Rates of oxygen uptake by washed cell suspensions were measured for all experiments except the experiments with 1000 mg/l inflow substrate concentration without recirculation. These values are presented in Table XII. The unit activity exhibits a decreasing trend at increasing values of dilution rate, indicating a smaller fraction of active mass at higher values of D. However, it has already been shown that insofar as substrate removal rate is concerned, this value increases with dilution rate, indicating that the cells are more active at higher dilution rates. These two results seem to contradict each other. It appears

TABLE XII

SLUDGE COMPOSITION AND UNIT ACTIVITY OF CELLS

Dilution Rate Hr^{-1}	S_i = 1000 mg/l Glucose					S_i = 3000 mg/l Glucose		
	Without Recir.		With Recir.			Without Recirculation		
	% Protein	% Carb.	% Protein	% Carb.	Unit Act.	% Protein	% Carb.	Unit Act.
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
1/24	57.65	13.13				51.70	10.66	6.3
1/18	60.22	9.80				60.00	13.33	8.13
1/12	63.40	11.15				53.10	11.67	10.60
1/6	62.20	22.10	59.85	28.37	2.095	61.00	22.50	7.38
1/4	59.37	24.50	54.36	32.10	1.104	-	-	-
1/3	42.30	32.33	61.22	34.32	1.350	49.98	28.75	5.77
1/2	40.75	31.22	52.75	32.66	2.54	42.30	29.70	1.00
1/1.50	39.30	35.76				41.70	31.30	0.532
1						38.40	34.90	0.362

that the specific substrate removal rate is a better measure of active cells than specific oxygen uptake rate. Since the presence of storage products within the cell can alter oxygen uptake values, the rate of removal of exogenous substrate per unit weight of cells is a better indicator of active fraction of cell mass. From this discussion it may be concluded that the active fraction of cell mass increases with dilution rate.

8. Sludge Composition

Only protein and carbohydrate content of the cells were measured in this study. The analyses were done on the samples collected at the end of the operation at each dilution rate. These results are presented in Table XII. It can be seen from these values that the protein content remained more or less constant at low values of dilution rate; however, there was a gradual increase in the carbohydrate content with increasing values of dilution rate (10 to 20 per cent). When washout of culture began in the system, the protein content decreased from about 50 per cent to 40 per cent. A corresponding increase in carbohydrate content was also observed. At low dilution rates, the increase in carbohydrate content was less (from 10 per cent to 20 per cent) and this may be attributed to the differences in storage products within the cell. At a dilution rate of 1/24 per hour, the carbohydrate content was 10 per cent. This indicates that almost all of

the stored carbohydrate has been utilized by the cell for maintenance and growth, since at this dilution rate the rate of supply of feed is very low.

When the mean residence time decreased below 3 hours, the protein content of the cells began to decrease with a corresponding increase in carbohydrate content. This may be explained on the basis that the organisms do not stay in the reactor sufficiently long enough to increase the protein content, and the carbohydrate content increases in the system, indicating glucose was removed from the medium and stored as carbohydrate before the protein synthesis had reached the peak value. If the organisms flowing out of the reactor were allowed to grow for a longer time, probably the protein content might have increased to the normal value. The above statement is only speculation, and no tests were conducted to support it. It may be concluded from the above discussions that the composition of sludge changes with dilution rate in a definite pattern, but no mathematical expressions were possible to describe it. Herbert (94) has reported that the composition of cells is a variable and any statement concerning cell composition without referring to the environment is virtually meaningless. Herbert (94) has also reported a decrease in protein content of cells at high dilution rates. His results are based on experiments conducted with Aerobacter aerogenes with glycerol as growth limit-

ing substrate. He has explained the decrease in protein content as due to the increase in RNA content of the cell. Since the RNA content of the cells was not measured in the present study, it is impossible to arrive at a definite conclusion. With respect to carbohydrate content, Herbert (94) has reported a constant value at all dilution rates in the case of Torula utilis grown with limiting carbon source. This result differs from the present study wherein the carbohydrate content increased with increasing dilution rate. Further studies are required along these lines before a definite conclusion can be reached.

9. Other Physical Parameters

Measurements of temperature, pH, and dissolved oxygen in the aerator at different dilution rates indicated that they remained more or less constant. The temperature was maintained at 25 ± 2°C., and variations in pH were negligible. However, it is important to point out here that it was possible to maintain the pH only by changing the amount of buffer added. When the mean residence time was less than 3 hours, the pH in the system decreased to 6.4 and the buffer concentration was doubled in order to hold the pH at approximately 7.0. Since it was decided to conduct all experiments at the same pH, the above procedure was adopted. The decrease in pH at low mean residence times indicates the release of metabolic intermediates that are acidic in nature. A preliminary anal-

ysis of the effluent samples revealed the presence of acetic acid at low detention times. The determination of dissolved oxygen at the various dilution rates indicated that the dissolved oxygen in the reactor was never less than 90 per cent of the saturation level. Therefore it may be concluded that none of the above parameters affected either the efficiency of substrate or COD removal, or introduced fluctuations in solids concentration.

In summary, the continuous flow, completely-mixed activated sludge process seems to be very promising from the standpoints of economy and biochemical efficiency. The economic considerations are based on the ability of the system to accept higher organic loadings at lower detention times, without a detrimental effect on the efficiency of substrate removal, than in the conventional activated sludge process. The kinetics of the completely-mixed process can be well represented by the equations proposed by Monod, provided the physiological growth parameters are determined at each dilution rate. The yield coefficients in continuous flow experiments were always higher than those obtained from batch experiments. Also, the above equations depict the solids and substrate levels only at dilution rates lower than that at which dilute-out begins. Further studies are required to investigate the differences between experimental and theoretical values during washout of the culture. Recirculation of sludge does not seem to enhance

the substrate removal efficiency of the system at the loading levels investigated. Krishnan (118) has also reported from his studies on quantitative shock loading that the systems without cell feedback responded successfully at certain dilution rates (D = 1/8 and 1/12 per hour). However, he has reported that in systems without cell feedback metabolic intermediates were released; whereas in systems with recirculation no significant amounts of intermediates were released. Therefore, it might be concluded that where severe shock loads are not expected, a completely-mixed system can be operated successfully, so far as substrate removal is concerned, without cell feedback.

CHAPTER VII

CONCLUSIONS

1. The results of the present study indicate that the relationship between rate of growth and concentration of substrate is represented better by Equation 14, proposed by Monod, than by any other relationships tested.

2. Chemical oxygen demand can be used as a measure of substrate concentration, provided the substrate used and the metabolic intermediates are known to be bio-degradable.

3. The physiological growth parameters obtained from batch experiments are not constants, but vary with dilution rate. These variations can be attributed to a selection of species imposed on the system by the hydraulic flow rate.

4. The relationship between growth and substrate consumption (yield) is also a variable, and these variations with dilution rate seem to be a function of maintenance requirement, rate of death of cells, and changing predominance of species.

5. Comparison of growth kinetics of mixed cultures in batch systems with that of pure cultures indicates that they are similar.

6. Steady states with respect to substrate concentration are subject to only minor fluctuations. However, fluctuations in biological solids concentration are distinct. The fluctuations in biological solids concentration can be attributed to changing yield coefficients.

7. COD loadings up to 53 lbs. per day per lb. of suspended solids can be treated by the completely-mixed process with a biochemical efficiency of removal greater than 90 per cent.

8. It appears that, from a biochemical standpoint at the loadings herein used, recirculation of sludge does not offer advantages with respect to efficiency of COD removal. However, sludge settleability is greatly enhanced by sludge recycling.

9. It seems feasible to operate a "closed" continuous flow system without sludge wasting, but the fluctuations in the concentration of biological solids are more distinct. In the closed system studied, the concentration of biological solids rose to 3600 mg/l and then decreased to 2000 mg/l; thereafter the solids level cycled within this range.

10. The per cent volatile content of the sludge in a total recirculation system did not decrease; it remained relatively constant with a mean value of 86.90 per cent.

11. There appears to be a relationship (Equation 97) between rate of oxygen uptake and rate of growth.

12. Long term experiments are necessary to obtain a reliable estimation of "steady-state" parameters; this is particularly true for the concentration of biological solids which always exhibited oscillatory changes.

13. It appears that changes in yield coefficient with dilution rate can be described mathematically by Equation 87 proposed by Hetling, et al. (15). According to this, the change in yield coefficient seems to be a function of maintenance requirement and rate of death of cells. However, it should be noted that the influence of changing predominance was not measured in the present study, but it could also contribute to the observed changes in yield coefficient.

14. Protein and carbohydrate content of the sludge, harvested at the end of each dilution rate experiment, showed little variation until washout of culture began. Further increase in dilution rate resulted in decreasing protein content (60 to 40 per cent) and increasing carbohydrate content.

15. The present study indicates that the active fraction of cell mass can be better estimated by specific substrate utilization rate than by specific oxygen uptake rate.

CHAPTER VIII

SUGGESTIONS FOR FUTURE WORK

1. It is more desirable to measure rate of growth by a method other than optical density. Oxygen uptake may provide a more accurate and easier method of measuring rate of growth. Further work is necessary along these lines to relate oxygen uptake and rate of growth. It is also desirable to relate viable count, mass of cells, and other metabolic processes to rate of growth.

2. In order to describe completely a continuous flow completely-mixed activated sludge process it is necessary to investigate the probable reasons for the tailing-off of the washout curve beyond certain values of dilution rate.

3. Since most waste-waters contain more than one organic compound as substrate, it is important to study the effect of multi-substrate wastes on various process relationships.

4. It is evident that most waste-water treatment plants are subject to both qualitative and quantitative shock loading. A large amount of work has already been conducted in the bioengineering laboratories of Oklahoma State University on the biochemical response of the sys-

tem to shock loading. Studies are warranted to obtain kinetic relationships for describing the completely-mixed process during periods of shock loading using experimental data already available.

5. The phenomenon of oscillation during steady state operation and the factors causing it need to be studied using either pure cultures or mixed cultures of known organisms in order to understand completely the behavior of the system.

6. Rheological properties (flow properties) of mixed liquor in a continuous flow completely-mixed system and their effects on oxygen transfer rate should be studied. It might also be possible to relate the rehological properties (e.g., viscosity) with growth of microorganisms.

7. Further investigations on the variations in sludge composition at various dilution rates and during shock loadings are warranted so that the biochemical response of the system can be evaluated.

8. It may be expected that certain wastes can be treated only by multi-stage completely-mixed reactors. Therefore, it is important to extend the present kinetic relationshipts to multi-stage reactor systems.

9. In order to make reuse of water possible, the complete removal of foreign materials from water becomes necessary. The possibilities of using tertiary treatments together with completely-mixed reactors should be investigated.

10. Since the growth parameters reported in this research work were obtained at low initial solids concentration, it will be of interest to study the effect of higher initial solids concentration on growth parameters.

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APPENDIX

LIST OF SYMBOLS AND ABBREVIATIONS

a - fraction of BOD removed for synthesis

a' - conversion factor between COD and volatile suspended materials

b - fraction of MLVSS used for endogenous respiration

B - growth parameter similar to k_s

B' - specific rate of substrate consumption for basal metabolism, hr^{-1}

B_1 - fraction of dead cells that lyse

B_2 - amount of substrate released per unit weight of lysed cells

B_3 - specific death rate of cells, hr^{-1}

c - concentration factor for return sludge

c' - an assumed constant that combines the conversion factor between COD and VSS (a') and the fraction of substrate removed that is synthesized

C - concentration of dye in the reactor at any time t, mg/l

C_i - concentration of dye in the inflow, mg/l

C_0 - concentration of dye at zero time, mg/l

C_2 - carbon content of substrate expressed as percentage

D - dilution rate; ratio between rate of flow (F) and volume of the reactor (V), hr^{-1}

E - specific endogenous respiration rate, hr^{-1}

f_o - fraction of substrate oxidized to CO_2 and water

F - rate of flow of nutrient solution through the reactor, ml/hr

ΔF_{ox} - free energy released by oxidation of one mole of substrate, kcal

i - concentration of impurities that serve as substrate, mg/l

k_c - specific catabolism rate which is equal to rate of substrate utilization per unit weight of cells, hr^{-1}

k_o - concentration of substrate at which $\mu_o = 1/2 \ \mu_{om}$

k_s - saturation constant

k_1 - rate constant relating rate of substrate removal and concentration of substrate

k_2 - rate constant describing the relationship between growth rate and substrate concentration

k_4 - constant of proportionality in units of gm/mole of ATP

k_5 - constant of proportionality relating moles of ATP and free energy released

k_6 - constant of proportionality relating free energy released and theoretical oxygen demand of one mole of substrate

k_7 - constant of proportionality relating carbon content, COD, and f_o

K - rate constant for substrate consumption when substrate consumption is a function of x and S

L - retention factor for suspended solids

$\bar{L}$ - concentration of 5-day BOD ($20^oC.$) remaining at steady state, mg/l

L_e - BOD value of stationary phase in a batch culture, mg/l

L_i - initial BOD, mg/l

n - number of generations

N_{ATP} - moles of ATP produced per mole of substrate

o - concentration of dissolved oxygen at any time t

p - factor relating rate of substrate utilization and rate of oxygen uptake

p' - probability of occurrence of an event

P - concentration of products other than cellular material, mg/l

P' - equal to $D(1 + \alpha - \alpha c)$

q - exponent of S in the equation proposed by Borzani, et al. (72)

r - fraction of substrate removed for respiration to produce energy for synthesis

r_1 - substrate transfer rate

s - fraction of substrate removed for synthesis of new cellular material and endogenous respiration

s_r - specific substrate utilization rate expressed as

rate of substrate utilization per unit weight of cells, hr^{-1}

s_{rm} - maximum value of s_r, hr^{-1}

S - concentration of growth-limiting factor, mg/l

$\bar{S}$ - steady state value of substrate concentration, mg/l

S_i - concentration of substrate in the inflow to the reactor, mg/l

S_0 - initial concentration of growth-limiting factor, mg/l

S_R - concentration of substrate in the return sludge, mg/l

S_1, S_2, S_3. . . etc., - concentrations of substrate in a series of multi-stage reactors, mg/l

$\bar{S}_1$, $\bar{S}_2$, $\bar{S}_3$. . . etc., - steady state values of the concen-centration of substrate in a series of multi-stage reactors, mg/l

$\bar{t}$ - mean residence time; equal to the ratio between V and F

t_e - time required to reach stationary phase in a batch culture

Δt - interval of time considered

$\phi(t)$ - frequency function of the residence time of a particle in a reactor

T_d or G - doubling time or generation time

u - fraction of substrate removed for storage within the cell

U - value of S at which $s_r = 1/2\ s_{rm}$

v - non-viable constant or specific mortality rate, hr^{-1}

V - volume of the reactor

x - concentration of biological solids in the reactor expressed as mass per unit volume, mg/l

x_a - average cell concentration over an interval of time, mg/l

x_d - concentration of dead cells, mg/l

x_e - concentration of biological solids in the effluent

x_i - concentration of biological solids in the inflow to the reactor, mg/l

x_0 - initial concentration of biological solids, mg/l

x_{nv} - concentration of non-viable cells, mg/l

x_v - concentration of viable cells, mg/l

x_A - concentration of active cell mass, mg/l

x_L - limiting concentration of biological solids that will be supported by the substrate present in the system, mg/l

x_{max} - maximum value of x, mg/l

x_1, x_2, x_3. . . etc., - concentration of biological solids in a series of multi-stage reactors, mg/l

$\bar{x}$ - steady state value of the concentration of biological solids in a single reactor, mg/l

$\bar{x}_1$, $\bar{x}_2$, $\bar{x}_3$. . . etc., - steady state values of the concentration of biological solids in a series of multi-stage reactors, mg/l

$\bar{x}_{11}$, $\bar{x}_{22}$. . . etc., - steady state values of the concentration of biological solids at different times t_1, t_2, etc., or at different dilution rates D_1, D_2, etc., mg/l

Y - yield coefficient expressed as mg solids produced per mg COD disappearance

Y_t - true yield coefficient

Y_B - yield coefficient obtained from batch experiments

Y_C - yield coefficient obtained from continuous flow experiments

z - theoretical oxygen demand of one mole of substrate

α - recirculation factor

α' - rate constant relating substrate consumption and u

α_1, β, and γ - constants relating rate of substrate utilzation, rate of growth, and rate of product formation

α_2, and β_2 - constants relating rate of product formation, rate of growth, and concentration of biological solids

λ - exponent of S in the specific growth rate equation proposed by Moser (19)

σ - rate of production of any product during transient state

μ - specific growth rate, hr^{-1}

μ' - equal to μ/Y, hr^{-1}

μ_m - maximum value of μ, hr^{-1}

μ_o - specific oxygen uptake rate, hr^{-1}

μ_{oi} - specific oxygen uptake rate due to presence of impurities, hr^{-1}

μ_{om} - maximum value of μ_o, hr^{-1}

μ_{app} - apparent value of μ, hr^{-1}

μ_1, μ_2, μ_3. . . etc., - values of specific growth rate in a series of multi-stage reactors, hr^{-1}

ATP - adenosine triphosphate

BOD - biochemical oxygen demand

COD - chemical oxygen demand

DNA - deoxyribonucleic acid

RNA - ribonucleic acid

MLSS - mixed liquor suspended solids

MLVSS - mixed liquor volatile suspended solids

VSS - volatile suspended solids

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